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Kidney Glycolipids in Late Infantile Metachromatic Leucodystrophy

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Late infantile metachromatic leucodystrophy is a sphingolipidosis characterized by the accumulation of large amounts of sulfatides in various organs notably neural tissue kidney and gall bladder [1–4] In addition, there is an increased excretion of sulfatides in urine the amount of which appears to be correlated with the activity of the pathological process [5]. The nature of the metabolic aberration in the disease is still unknown as well as the biosynthetic and catabolic pathways of sulfatides under normal conditions. Jatzkewitz [6] has proposed that the sulfatides are precursors of the cerebrosides and that in metachromatic leucodystrophy there is a lack or defect of a cerebroside sulfatase Recently he [7] has presented evidence for a decreased activity of kidney cerebroside sulfatase from patients with this disease This hypothesis is supported by the fact that in brain there is a striking, although incomplete decrease in the cerebroside fraction [8]. For the kidney in which organ the lack of cerebroside sulfatase was demonstrated, no data are available about the concentration of cerebrosides, only figures for neutral glycolipid hexose [4 8] Therefore a detailed study of the kidney glycolipids in normal and affected children was undertaken as part of an investigation on the biochemistry of late infantile metachromatic leucodystrophy [9].

Materials and Methods

Materials Normal human kidneys were obtained from the Institut of Pathology I Sahlgren Hospital, Gothenburg. They were taken from four cases aged 10–17 years with no known kidney disease The kidneys were removed at autopsy within 4 hours of death, and then stored at −20°C for 1 2 years. Representative samples of all kidneys were prepared together

Kidneys from four cases with metachromatic leucodystrophy were available. Their clinical data have recently been described by Hagberg [10] and are summarized in Table 1. The autopsies were performed within 12 hours after death and the organs immediately frozen. Representative samples of kidney tissue were dissected within a week, homogenized and lyophilized, and kept at −20° pending analysis (from 1 to 2 years). One kidney from patient R. A., the most fulminant case, was used for large-scale preparation of glycolipids. The kidney had been stored for three years at −20 in the fresh state.

Adsorbents for chromatography included silicic acid (Mallinckrodt A. R.) 100 mesh,

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diethylaminoethyl (DEAE) cellulose (Whatman powder DE 50, W. & R. Balston Ltd.), cellulose (Schleicher and Schüll, No. 123B) and Silica Gel G (E. Merck A. G., Darmstadt). Organic solvents were of analytical reagent quality and were distilled before use.

Analytical methods. Thin-layer chromatography (TLC) was carried out on Silica Gel G plates 0.25 mm in thickness which were made with equipment produced by C. Desaga GmbH, Heidelberg, Germany. The solvents used were chloroform-methanol-water (65:25:4, v/v/v) for the neutral glycolipids and sulfatides, chloroform-methanol-5 N ammonia (60:35:8, v/v/v) for separation of the sulfatides [11] and chloroform-methanol-water (60:35:8, v/v/v) and n-propanol-water (7:3, v/v) for separation of the gangliosides [12, 13]. The neutral glycolipids and the sulfatides were detected by spraying with 50% sulfuric acid [14]. The gangliosides were detected by spraying with the resorcinol reagent [15].

Paper partition chromatography of the sulfatides was performed on silicic acid impregnated paper (Schleicher and Schüll, No. 289) after methods described by Marinetti [16] using the solvent system diisobutyl ketone-acetic acid-water (40:20:3, v/v/v). The sulfatides were then detected by dipping the paper in cresyl violet [4] or Rhodamine 6G. The IR spectra were recorded in a Perkin-Elmer Infracord Spectrophotometer.

Hexoses [17], hexosamine [18] and sialic acid [19] were determined as described by Svennerholm. Sulfate was determined by the method of Mårtensson [20]. Lipid phosphorus was analysed with a micro modification of the Lowry method [21]. The fatty acid composition was analysed according to Stållberg-Stenhagen and Svennerholm [22]. Qualitative sugar TLC was performed as in the method of Kuhn and Wiegandt [23] after hydrolysis of the lipids in 2 N HCl at 100°C for 2 hr.

Isolation of the glycolipids. Mini preparation. 200 mg of freeze-dried kidney was extracted with 25 ml chloroform-methanol (2:1, v/v) (C-M 2:1) in a small Soxhlet apparatus for 4 hr and then with the same amount of C-M 1:2 overnight. The combined extracts were evaporated to dryness in vacuo in a rotating evaporator at 40°C. The extracted lipids were then dissolved in 5.5 ml methanol and 0.5 ml 2 N KOH was added. The flask was stoppered and hydrolysis was allowed to proceed in a water bath at 37°C for 16 hr. The hydrolysate was neutralized with 1 N HCl using methyl red as indicator and was then made slightly alkaline with one drop of N KOH. The lipids were extracted from the hydrolysate by adding 4 vol of C-M 2:1, shaking vigorously for 1-2 min, and then centrifuging at 3000 rpm for 10 min. After discarding the upper phase, the lower phase was taken to dryness, dissolved in about 1 ml chloroform and added to a 1 g column of silicic acid. The column was eluted with 15 ml chloroform and then with 15 ml C-M 2:1. This last fraction contained all the neutral glycolipids and the sulfatides. They were separated by chromatography on a 1 g column of DEAE cellulose in a 9 × 1 cm. The neutral glycolipids were first eluted with 25 ml C-M 2:1 and then the acid glycolipids with 25 ml 0.5 M LiCl in C-M 2:1 containing 10% H_2O. After overnight dialysis of the lithium chloride eluate, both fractions were taken to dryness and dissolved in a small volume of C-M 2:1. One half of the amount was analysed by TLC.

Maxi preparations. The large scale preparation was accomplished according to methods previously used by Mårtensson [24] for the isolation of human kidney glycolipids with the following three exceptions: (1) unfixed deep frozen tissue was used; (2) sufficient N KOH was added during the homogenization and the subsequent extraction to maintain a pH greater than 7.5 in order to prevent a possible loss of the more complex acid glycolipids, the gangliosides, and (3) the lipid extracts were chromatographed on a cellulose column prior to alkaline hydrolysis in order to remove non-lipid contaminants and to separate the more complex and labile gangliosides from the other lipids.

The kidneys were freed from capsules and

TABLE 1 *Age at onset and at different clinical stages, and the cl ical picture shortly before death.*

Patient	First symptoms at	Clinical stages I	II	III	IV	Clinical picture shortly before death
	Age in years					
A. A.	1½	1½–1½	1½–2½	2½–3	Died before this stage	Moderately retarded, variable rigi dity no bulbar igns poor vision, positive peripheral nerve biopsy positive urine sulfatide test CSF protein 81 mg/100 ml
A. L.	1½	1½– ½	2½–3	3–3½	3½–3½	Stuporous, decerebrat rigidity bulbar signs present, blind, optic atrophy positive peripheral nerve biopsy positive urine sulfatide test CSF protein 150–190 mg/100 ml
R. A.	1½	1½–2	2 ½	2½–2½	½–4½	
I. M.		2–4½	4½–5	5–5½	5½–7½	

Clinical stages.
I Ability to walk or to stand with or without support
II Inability to stand but able to sit with or without support.
III Bedridden without ability to stand or to sit but with some voluntary movements.
IV Bedridden without bility to perform any voluntary movements and without contact with the surroundings.

int small pieces and washed several times with 0.9% saline t remove blood. They were homogenized in the presence of KOH with 1 volume of C–M 1 1 in a Turmix blendor and then another 4 volumes of the same solvent mixture was added together with additional 1 N KOH After standing overnight, the extract was filtered and the residue was extracted with boiling C–M 2 1 for 30 minutes. The combined extracts



Fig. 1 Thin layer chromatography of neutral kidney glycolipids from micropreparations. W F = Normal; A. A. A. L. I. M. and R. A. = cases with metachromatic leucodystrophy 1, ceramide-monohexosides, 2, dihexosides; 3, tri hexosides; 4 aminogl colipids Solvent Chloroform-methanol-water (65 25 4) spraying reagent: 50 sulfuric acid

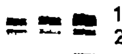


Fig — Thin layer chromatography of kidney sulfatides from micropreparations. W F., Normal; A. A. A. L. and R. A., cases with metachromatic leucodystrophy *1* Monohexose-sulfatides; *2* dihexose-sulfatides. Solvent Chloroform-methanol-water (65 25 4 /v/v); spraying reagent. 50 sulfuric acid.

TABLE 2. *Concentration of neutral glycolipid and sulfatide hexose in kidney*

The hexose figures are given in mg/g dry tissue weight

Patient	Normal	A.A.	A.L.	L.M.	R.A.
Neutral glycolipid hexose	1.8[a]	2.8	_3	3.0	_4
Sulfatide hexose	0.1	_8	_8	6.8	8.6
Percentage of water	—	82.1	82.0	81.7	82_

[a] Value determined from isolated neutral glycolipids in large-scale preparation.

were then chromatographed on a cellulose column [25] The glycolipids now freed from the more complex gangliosides were then isolated as described for senile kidney [24]. The steps in this procedure consist of a mild alkaline hydrolysis, silicic acid chromatography to remove fatty acids, released by the alkaline hydrolysis, and sphingomyelin DEAE-cellulose chromatography to separate the neutral and acid glycolipids, and a final purification of the glycolipid fractions by rechromatography on silicic acid columns and preparative thin layer plates.

From the normal material (ca. 185 g wet weight) the individual neutral glycolipids could be isolated separately; however there was insufficient material from the pathological case (ca. _5 g wet weight) to separate the constituent neutral glycolipids The isolated glycolipids were analyzed for hexose sulfate and phosphorus. The sugars were identified by thin layer chromatography The IR spectra of the sulfatides were obtained after precipitation of the lipids from methanol. Finally the sulfatide fatty acid composition was determined from chromatographically pure unprecipitated compounds.

Results

Micropreparations The pattern of the neutral glycolipids is shown in Fig 1 The first case represents normal kidney the remaining four are from diseased children in order of increasing severity of the process. The general pattern and the amount of lipid bound hexose (Table 2) are similar in each instance The cerebroside fractions in particular shows very little variation.

Fig _ represents the kidney sulfatide patterns The increase in this fraction is apparent in the diseased children. As earlier described from senile kidney [26], two sulfatide fractions can be seen, the first being monohexose-sulfatides (sulfuric acid ester of *n*-acyl-sphingosine galactose) and the slower moving component being dihexose-sulfatides (sulfuric acid ester of *n* acylsphingosine glucose-galactose) In the diseased group, the monohexose-sulfatides appear to have increased relative to the dihexose-sulfatides

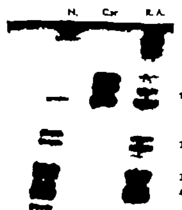


Fig 3. Thin layer chromatography of isolated neutral glycolipids. N Normal; R. A. case with metachromatic leucodystrophy *Cer* reference mixture of 50 µg glucocerebroside and 50 µg galactocerebroside *1* ceramide-monohexosides (cerebrosides); *2*, dihexosides; *3*, trihexosides *4* aminoglycolipids. (N represents 80.8 µg lipid-hexose and R. A. 82.1 µg lipid-hexose) Solvent Chloroform-methanol-water (65 _5 4 v/v) spraying reagent 50 _ sulfuric acid.

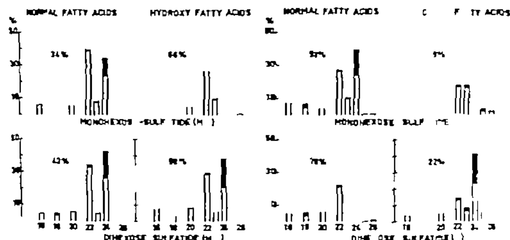


Fig. 4. F tty acid content of kidney glycolipids. *ML*, metachromatic leucodystrophy; \ normal. □ saturated fatty acid; ■ mono-unsaturated fatty acid.

Case R. A. revealed a very high sulfatide content and was selected for a detailed study of the kidney glycolipids.

Macropreparations In Fig. 3 the neutral glycolipids from the normal and pathological kidneys can be compared at TLC As in the micropreparation the two patterns are similar In the case R. A. the amount of galactocerebroside is no less than in the normal.

The kidney sulfatides isolated from the pathological kidney represented an increase of 70-fold when compared with the normal kidneys and, in this instance composed 5 0 % of the dry tissue weight From the normal material the amount of monohexose-sulfatides was 0.54 mg/g and of dihexose-sulfatides 0 20 mg/g dry tissue weight· corresponding results from case R. A. were 40.2 mg/g and 0.4 mg/g dry weight respectively The sulfatides from each case yielded the same R_F-value on TLC and silicic acid impregnated paper chromatography as compounds earlier isolated from senile kidney [26] In addition, the IR-spectra of the sulfatides from the pathological case were identical with those from the senile kidney [26]

Fig 4 illustrates the fatty acid content of the two sulfatide fractions from the case R A. as compared with those of normal kidne These lipids are normally characterized by their high content of C_{22} and C_{24}-fatty acids. The sulfatides of R. A differed from those of normals by (1) a lower content of monounsaturated acids, ( ) a higher content of hydroxy acids and (3) a remarkably high content of C_{22}-acids The dihexose-sulfatides of both normals and case R A. contained less hydroxy acids, and the content of normal C_{24}-acids and of C_{24}-hydroxy acids was lower than in the monohexose-sulfatides.

The gangliosides from the normal and pathological materials are compared in Fig 5 In the normal preparation there were only very small amounts of di- and trisialogangliosides In the dominating monosialoganglioside fraction, a compound with the same R_F-value as n-acyl-sphingosine-glucose-galactose-sialic acid, GM_3, was most prominent, but there

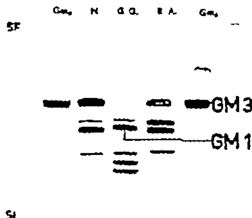


Fig 5. Thin layer chromatography of gangliosides. GM_3, n Acylsphingosine-glucose-galactose-sialic acid; GG gangliosides from normal cerebral gray matter; N gangliosides from normal kidney; R. A., gangliosides from metachromatic leucodystrophy kidney Solvent Chloroform–methanol–water (60 35 8 v/v/v); spraying reagent: resorcinol–HCl.

were also appreciable amounts of a compound with a slightly lower R_F-value than monosialogangliotetrose GM_1 of brain. The exact sequence of the sugars of this kidney ganglioside cannot be stated There were also small amounts of a third ganglioside running between the other

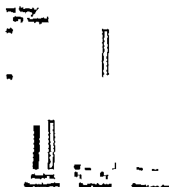


Fig 6. Kidney glycolipid profiles all concentrations expressed in mg/g dry tissue weight. Normal, ■ metachromatic leucodystrophy; S_I, monohexose-sulfatides; S_{II}, dihexose-sulfatides; Neutral glycolipids = lipid bound hexose 2.7; gangliosides lipid-bound sialic acid 4

two probably a monosialogangliotriose

In the case R. A the GM_3 fraction was decreased. Instead there was an increase of the proposed monosialogangliotriose and of disialogangliosides

Fig 6 summarizes the concentrations of the three glycolipid fractions isolated from the normal and pathological material. The similarity in the total neutral glycolipid and ganglioside fractions is contrasted with the marked increase of the sulfatides.

Discussion

In the present cases of late infantile, metachromatic leucodystrophy the total amount of sulfatides accumulated in the kidneys represented an enormous increase, 25- to 70-fold when compared to normal juvenile kidneys In cerebral white matter site of the most severe morphological changes the increase of sulfatides was not more than 7 fold [8, 27] Austin [4] has earlier determined the sulfatide concentration in kidneys and cerebral white matter from patients with metachromatic leucodystrophy as compared to normals. He found much smaller increases of sulfatides in both kidney and cerebral white matter than we, 9-fold and 2 to 3-fold, respectively With regard to the kidneys, the figures for sulfatides in the pathological material are about the same in the two studies but his normal values are 5 to 10 times higher than ours. The present figures for sulfatides were obtained from hexose and sulfate determinations on chromatographically purified lipid extracts [17] and from the nearly quantitative isolations of sulfatides [26]. Austin [4] determined sulfatides by infrared spectrometric analysis of partially purified lipid ex

tracts. It is evident that this procedure is liable to very large errors when applied to tissues with a low sulfatide content.

The kidney sulfatides were defined as *N*-acyl-sphingosine-galactose sulfuric acid and *N*-acyl-sphingosine-glucose-galactose sulfuric acid, i.e. the same as those normally present [26]. The relative proportions between mono- and dihexose-sulfatides were different—4.3 : 1 in case R. A. and 2.6 : 1 in the normal kidneys. The small-scale preparations show that the ratio between the sulfatides is about the same in all the pathological cases, and clearly different from the normal. It is necessary however to note that the proportion between the two sulfatides in senile kidney is 8 : 1 [26]. While the monohexose-sulfatide concentration was about the same in juvenile and senile human kidneys, the dihexose-sulfatide concentration decreased in older ages.

In comparing the neutral glycolipids as well as the gangliosides from normal and pathological material, the concentrations of the respective fractions were nearly equal. However TLC revealed definite variations in the ganglioside patterns. Thus during the comparison of specific tissue components one has to consider both the figure for the total amount and the relative proportions of individual *subcomponents. The observed* deviations in the ganglioside pattern are likely to be secondary to the disturbed sulfatide metabolism and can thus be interpreted as expressing the intimate interrelationship in the metabolism of different glycolipids.

The *fatty acid analyses of the sulfatides* from the case R. A. revealed certain differences from the sulfatides normally present in human kidneys, namely increase of the hydroxy acids, increase of the normal C_{22}-acids and decrease of unsaturation. These deviations from the normal fatty acid pattern are difficult to explain. One possible mechanism is that the changes are due to disturbed physiological conditions by which sulfatides with a fatty acid composition deviating from normal will be synthesized. In an attempt to evaluate this problem the sphingomyelins from the normal and the pathological material were analysed for their fatty acid composition. In the latter a decreased saturation of C_{24}-acids and a small but significant increase of the C_{22}-fraction was found. These changes have also been observed in brain sulfatides and cerebrosides in this disease as well as in two cases of severe circulatory and respiratory insufficiency [9].

Another possibility is that the accumulated kidney sulfatides are largely biosynthesized in another organ, and then transported to the kidneys. The fatty acid composition of the kidney sulfatides would then reflect the sulfatide fatty acid composition in the organ from which they were derived. The most likely source would, of course, be the brain, which has a far higher concentration of sulfatides than any other organ. This possibility can, *however* be directly excluded, as the accumulated kidney sulfatides have a quite different fatty acid composition from brain sulfatides both in normal and metachromatic leucodystrophy cases. But the possibility still remains that they are derived from other organs.

The metabolic pathways for cerebrosides and sulfatides are still incompletely known [28]. It has been shown that cere-

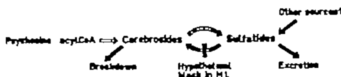
brosides can be synthesized from psychosine (sphingosine-galactose) and acylCoA [28] and from incorporation studies with ^{14}C-galactose it is likely that in brain, cerebrosides are precursors of sulfatides [29]

cerebrosides → sulfatides

Jatzkewitz [6] has, however suggested the normal pathway to be

sulfatides → cerebrosides

He has supported his hypothesis by the isolation of a sulfatide sulfatase from kidney and by the demonstration of sulfatase activity in many other organs, including brain [7] He has also presented evidence for a decreased activity of this enzyme in metachromatic leucodystrophy The available data can then be interpreted according to the following scheme



The fact that no absolute decrease of the kidney cerebrosides could be demonstrated in the present study will not exclude the possibility of a sulfatase defect in this disease It shows rather that the hydrolysis of the sulfatides is no major pathway in the formation of kidney cerebrosides.

The increase of the kidney sulfatides in metachromatic leucodystrophy also involves the dihexose-sulfatides, which suggests that the two sulfatides should be hydrolysed by the same enzyme system The dihexose-sulfatides are desulfated to ceramide-dihexosides. This pathway will probably only to a small extent contribute to the formation of the ceramide-dihexosides and it was noted that this fraction also remained unchanged in the metachromatic leucodystrophy cases

Summary

1 Micropreparations from kidneys of children with metachromatic leucodystrophy were analyzed for neutral glycolipid and sulfatide patterns

2 Sulfatides neutral glycolipids, and gangliosides were isolated from a single case of metachromatic leucodystrophy and from a pooled sample of normal kidneys.

3 The sulfatide content of the pathological material was increased 70-fold over normal. The concentration of total neutral glycolipids and gangliosides were similar in normal and pathological material but definite variations in the ganglioside TLC pattern were detected.

4 Sulfatide fatty acid analyses differed from those of normal material There was an increase of the hydroxy acids and of the normal C_{24}-acids and a decreased degree of unsaturation. Possible mechanisms for these changes are discussed

5 Metabolic pathways of sulfatides and cerebrosides are discussed in relation to the disease mechanism

Acknowledgement

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References

1 Austin J H Metachromatic form of diffuse cerebral sclerosis I. Diagnosis during life by urine sediment examination. *Neurology 7* 415, 1957
2. Jatzkewitz, H.: Zwei Typen von Cerebrosid-Schwefelsäureestern als sog "Prälipoide" und Speichersubstanzen bei der Leukodystrophie, Typ Scholz (metachromatische Form der diffusen Sklerose). *Hoppe-Seyler Z Physiol Chem, 311* 279 1958.
3. Hagberg, B., Sourander, P Svennerholm L. and Vose, H.: Late infantile metachromatic leucodystrophy of the genetic type *Acta Paediat Scand, 48* 200, 1957
4 Austin, J H Metachromatic form of diffuse cerebral sclerosis. *Neurology 10* 470, 1960
5. Hagberg, B., Svennerholm, L. and Wharton, B.: The excretion of urinary sulfatides in health and neurological disease. *Acta Paediat Scand 54* 409, 1965.
6 Jatzkewitz, H Die Leukodystrophie Typ Scholz (metachromatische Form der diffusen Sklerose), als Sphingolipidose (Cerebrosid-Schwefelsäureester Speicherkrankheit). *Hoppe-Seyler Z Physiol Chem 318* 265, 1960
7 Mehl, E. and Jatzkewitz, H. Eine Cerebrosidsulfatase aus Schweinenniere. *Hoppe-Seyler Z Physiol Chem, 339* 80, 1965
8. Svennerholm L. In *Brain Lipids and Lipoproteins and the Leucodystrophies* (edited by Folch Pi and Bauer). Elsevier Publishing Co., Amsterdam, 1963, p. 104
9 Hagberg, B., Sourander, P and Svennerholm, L.: The biochemistry of late infantile metachromatic leucodystrophy (in preparation)
10. Hagberg B.: In *Brain Lipids and Lipoproteins and the Leucodystrophies* (edited by Folch-Pi and Bauer). Elsevier Publishing Co., Amsterdam, 1963, p. 134.
11 Muldner, H. G Wherrett J R. and Cumings, J N Some applications of thin layer chromatography in the study of cerebral lipids. *J Neurochem 9* 607 1962.
12 Wagner, H., Hörhammer, L. and Wolff P Dünnschichtchromatographie von Phosphatiden und Glycolipiden. *Biochemische Z, 334* 175, 1961
13. Kuhn R., Wiegandt H and Egge, H Structure of gangliosides. *Angew Chemie, 73* 580 1961
14. Morris, L. J Holman R. T and Fontell, K.: Naturally occurring epoxy acids. Detection and evaluation of epoxy fatty acids by paper thin-layer and gas-liquid chromatography *J Lipid R* 68, 1961
15. Svennerholm, L.: Chromatographic separation of human brain gangliosides, *J Neurochem, 10* 613, 1963
16. Marinetti, G V.: Chromatographic separation, identification, and analysis of phosphatides. *J Lipid Res, 3* 1 1963
17 Svennerholm, L.: The distribution of lipids in the human nervous system I Analytical procedure Lipids of foetal and newborn brain. *J Neurochem 11* 839 1964
18. — The determination of hexosamines with special reference to nervous tissue *Acta Soc Med Upsal, 61* 287 1956.
19 — Quantitative estimation of sialic acid. *Biochim Biophys Acta, 24* 604 1957
20 Mårtensson E.: Quantitative estimation of sulfates in lipid extracts. *Biochim Biophys Acta, 70* 1 1963.
21 Svanborg, A. and Svennerholm, L. Plasma total lipid cholesterol, triglycerides, phospholipids and free fatty acids in healthy Scandinavian population, *Acta Med Scand, 169* 43, 1961
22. Ställberg-Stenhagen S. and Svennerholm, L.: The fatty acid composition of human brain sphingomyelins Normal variation with age and changes during myelin disorders. *J Lipid Res, 6* 140, 1965.
23. Klenk, H and Mahera, G Zur dünnschichtchromatographischen Trennung einiger einfacher kinasch wichtiger Zucker in biologischem Material. *Schweiz Med Woch 94* 164, 1960.
24. Mårtensson E.: Neutral glycolipids of human kidney *Biochim Biophys Acta* (in press)
25. Svennerholm, L Composition of gangliosides from human brain, *Nature, 177* 524, 1956.
26. Mårtensson E. On the sulfatide containing lipids of human kidney *Acta Chem Scand 17* 1174, 1963.
27 Svennerholm, L.: *Lipidoses* In *Metabolism and Physiological Significance of Lipids* (edited by Dawson and Rhodes). John Wiley and Sons Ltd., London, 1964 p 553.
28. Brady R O In *Metabolism and Physiological Significance of Lipids*. John Wiley and Sons Ltd., London, 1964, p 95.
29. Hauser, G Labeling of cerebrosides and sulfatides in rat brain. *Biochim Biophys Acta 84* 1., 1964

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Glycogenosis Type 6 (Liver Phosphorylase Deficiency)

A Case Followed for Ten Years with Normal Phosphorylase Activity in White Blood Cells and Jejunal Mucosa

by P A. ÖCKERMAN H. JELKE and K. KAIJSER

Type 6 glycogenosis first described in 1959 by Hers [5], has been held to be the most common type of glycogenosis [7 9] The diagnosis of this disease is based on the clinical symptoms and signs on the histological evaluation and, ultimately on the biochemical analysis of liver tissue Recently it was claimed from two different laboratories that white blood cells have a low phosphorylase activity in cases of glycogenosis type 6 and that consequently a definite diagnosis can be made on this basis [8 17 18].

As a rule the clinical course of the disease has been found to be fairly mild but only few cases have been described in detail [3 7 1..]

Methods

Unless otherwise stated, the clinical chemical analyses in 1953 were performed at the Clinical Chemical Laboratory (Head: B. Uhnoo, M.D), Central County Hospital, Gävle using the same methods (not specified here) as in the ordinary routine analyses. This also applies to 1963, the routine analyses being made at the Central Laboratory Eskilstuna.

The determination in 1963 of plasma free fatty acids and glycerol [1], as well as of glycogen and enzymes in tissues [15], was as described earlier

Case Report

Heredity

Both parents are healthy and are not blood relations. Two brothers and one sister are clinically healthy

Clinical development of the patient Laboratory histological and biochemical findings

T E., male, born in 1948 at expected term. Birth weight 3200 g Birth and immediate postnatal period without complications. After a few months, the mother noticed that the patient had a prominent abdomen, but no investigation at hospital was performed. Motor development was somewhat late the patient being able to sit at 8 months and walk at 20 months. Mental development was normal, as judged by the mother No increased tendency to infections or bleeding was noted, nor any seizures. The appetite was found by the mother to be extremely large The patient sometimes complained of slight abdominal pains.

In 1953, at age 5, he was hospitalized because of encopresis. During this hosp

TABLE 1 *Laboratory data*

Routine haematological tests were normal (haemoglobin, red blood cell, white blood cells, differential counts of white blood cells in 1953 and 1963, reticulocytes and bone marrow examination in 1953). Routine urine tests for sugar and protein and urinary sediment were normal in 1953 and 1963. Routine liver function tests were normal (thymol turbidity, alkaline phosphatase and bilirubin in 1953 and 1963, and Weltmann reaction, citric acid in serum, Takata reaction and oral galactose tolerance test in 1953). Serum total proteins and non protein nitrogen in 1953 and bleeding time prothrombin time thrombocytes and capillary resistance in 1963 were normal. In 1953 the ECG and ophthalmo-neurological signs were normal.

	1953	1963
Blood glucose mg/100 ml	95–88–114–117–150–83–120–81	86–82[b] See also Table
Oral and intravenous glucose tolerance tests	Normal	Not tested
Insulin tolerance test (1 6 IU i.v.)	Blood glucose from 117 to 70 mg/100 ml in 20 min. Normal tolerance	Not tested
Legal acetone test in urine	Negative on 10 occasions. Traces on one occasion	Not tested in fasting state See also Fig. 1
Serum cholesterol mg/100 ml	420*–390*–310[c]* 270[c]* 285[c] –300*	196–164
Serum total lipids	1200* 1300[c] mg/100 ml	4.9–5.2 Kunkel units
ESR mm/1h	9–11 7–8–10	3
Serum enzymes		
GOT[d] (U/ml)	Not tested	22 –3
GPT[e] (U/ml)	Not tested	16–18
LDH[f] (U/ml)	Not tested	210–220
Serum uric acid mg/100 ml	Not tested	6

[a] Reductometric method [4]. [b] Glucose oxidase method [10]. [c] On fat-free diet. [d] Glutamic oxaloacetic transaminase [e] Glutamic pyruvic transaminase [f] Lactic dehydrogenase.

* Denotes values considered definitely pathological.

tallization, glycogen storage disease was diagnosed. The patient was significantly small for his age (according to the scale of Karlberg & Iggborn [11]) without tendency to obesity (Height 99 cm and weight 16.3 kg.) He was found to have remarkably large appetite. Small xanthomatous eruptions were present on the right supercilium. Laboratory investigations (Table 1) showed hyperlipaemia and hypercholesterolaemia, but no hypoglycaemia. Liver function tests did not indicate any damage to the liver parenchyma. The ESR was essentially normal. The insulin tolerance was normal. An



Fig. 1 Adrenalin tolerance tests. Dose not recorded in 1953, 1 mg s.c. in 1963. Blood glucose level analysed by reductometric method [4] in 1953 and by glucose-oxidase method [10] in 1963.

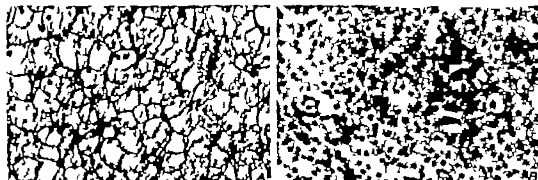


Fig 2. *Liver biopsy* A, 1953. Fixed in formalin. Van Gieson. Not the enlarged, strongly vacuolated liver cells, the cytoplasm of which forms fine network. The cells are filled with glycogen and t moderat degree with fat (R. Fåhraeus). B, 1963. Fixed in formalin. Van Gieson. Normal ppearance (H. Nordenstam).

adrenalin tolerance test showed a normal response in blood glucose (Fig 1)

Roentgenological examinations disclosed marked hepatomegaly the liver reaching the iliac crest, without associated splenomegaly At laparotomy for liver biopsy the liver was found to be massively enlarged, with a smooth surface, normal consistence and a definitely lighter colour than normal. The spleen was of normal size and consistence.

Histological examination of the liver biopsy cimen (Fig. *) indicated glycogen storage disease

The patient was in excellent health during his 3 months st y in hospital.

Between 1953 and 1963 he was seen at frequent intervals. The abdominal prominence diminished successively between the age of 5 and 10 and the contour has subsequently been normal Between the age of 5 and 15 he was in good health.

In 1963 at age 15 he was hospitalized again, although in excellent health with no complaints, after he had been found in a search for all cases of glycogen storage disease in Sweden by one of us [15]

Clinical examination on this occasion disclosed no signs of disease but slight hepatomegaly was found at roentgenological examination (8"0 ml/m body surface area)

The results of all laboratory tests were normal, except those for free fatty acids. It can be seen in Table 2 that the plasma level of free fatty acids was, on every sampling occasion, at or above the normal upper limit (as given in ref 14) Blood gl cose and plasma glycerol and free fatty acids were determined at intervals during a 24-hour period, during which an adrenalin tolerance test was also made It can be inferred from Table 2 and Fig. 1 that the blood glucose responded normally to adrenalin administration. Administration of adrenalin elicited an obvious response also in plasma free fatty acids and glycerol.

Histological xamination of liver biopsy tissue showed nothing abnormal (Fig. 2 B) (H. Nordenstam) On the other hand biochemical studies of two different specimens disclosed a high but not definitely pathological glycogen level, and a marked decrease in phosphorylase activity (T ble 3)

Normal phosphorylase activity was found in whit blood cells, as well as in jejunal mucosa (Table 3)

The glycogen level was raised in red blood cells (Table 3)

Regardless of the absence in 1963 of obvious clinical symptoms and signs, and despite the findings in whit blood cells, glycogen storage disease was once more diagnosed, and regarded to be of Cori's type 6.

TABLE 2. *Blood glucose and plasma glycerol and free fatty acids du a 4-hour period* Adrenalin tolerance test performed on March 29, 1963, t 1 p.m Fig 1).

Dat	Time	Nutritional status	Glucose mg/100 ml	Glycerol µMole/ml	Free fatty acids µEq ml
March 29 1963	8 a.m.	Fasting	—	0 08	1 12
	12 a.m.	Fasting	58	0.113	1 1
	1.30 p.m.	Fasting	109	0.1 2	1 49
		30 min after adrenalin, 1 mg			
	4 p.m.	F sting	7	0.099	1 [illegible]
	6 p.m.	Not fasting	75	0 085	1 4
March 30, 1963	8 a.m.	Fasting	72	0.069	1

Discussion

The only finding that according to current knowledge can be said to argue directly against a diagnosis of glycogenosis type 6 is the normal activity of phosphorylase in white blood cells. This activity may possibly be an expression of a similar hitherto unknown factor at work in some cases in which no direct relation could be established between reduced enzyme activity and glycogen deposition in th liver [6 7 9] When the result of the analysis on whit blood cells is weighed against the collected clinical chemical and histological findings in 1953 and the results of enzyme and glycogen assays on liver biopsy specimens and red blood cells in 1963–64 we cannot however draw any other conclusions than that our patient does with reasonable certainty represent a case of glycogenosis type 6

The assay of phosphorylase in a biopsy specimen of jejunal mucosa represents a new diagnostic approach In line with that tried successively in glycogenosis type 1 by one of us [13]. The finding of normal phosphorylase ctivity in jejunal mucosa precludes utilization of this type of biops for diagnostic procedures in at least some of the cases, suspected to have type 6 glycogenosis. It does not on the other hand, prove that jejunal phosphorylase always has a normal activity in cases of liver phosphorylase deficiency The latter argument is analogous to that relevant to the assay of phosphorylase in white blood cells (see the following section)

In recent reports on the phosphorylase activity in white blood cells in glycogenosis type 6 it has been stated that the relative decrease in activity was more marked than in the liver [8 17] It was also stated that a similar decrease in phosphorylase activity in white blood cells was found in one of the two parents in two families Important conlusions on the inheritance of type 6 glycogenosis were drawn from these results and from the fact that the enzym is not totally inactive in the liver The findings were thus considered to support the theory earlier launched by Hers [5] implying inheritance of the disease as an autosomal, dominant trait Our finding of normal activity f phosphorylase in white blood cells does not

References

1. Brante, G., Kaijser, L. and Öckerman, P. A. Glycogenosis type I (lack of glucose-6-phosphatase) in four siblings. *Acta Paediat Scand, Suppl 157* 1964.
2. Crosby, W. H. and Kugler, H. W.: Intraluminal biopsy of the small intestine. The intestinal biopsy capsule. *Amer J Digest Dis, 2* 236, 1957.
3. Gamillan, J. C. Les glycogénoses hépatiques. Corrélations entre les caractères cliniques et biochimiques et le type de déficit enzymatique: glucose-6-phosphatase amylo-1,6-glucosidase et phosphorylase. *Rev Internat Hepatol, 10* 699, 1960.
4. Hagedorn, H. C., Halström, F. and Jensen, A. Hurtige metoder til bestemmelse af blodsukker ved kaliumferricyanid. *Hospitalstid, 78* 1193, 1935.
5. Hers, H. G. Études enzymatiques sur fragment hépatiques. Application à la classification des glycogénoses. *Rev Internat Hepatol, 9* 35 1959.
6. — Recent developments in the biochemistry of glycogen storage disease and of fructose intolerance. *Chemisch Weekblad 57* 437 1961.
7. — Glycogen storage disease. *Advances in Metabolic Disorders 1* 1 1964.
8. Hulsman, W. C., Oei, T. L. and Van Creveld, S. Phosphorylase activity in leucocytes from patients with glycogen storage disease. *Lancet, ii* 581 1961.
9. Illingworth, B. and Brown, D. H.: Glycogen storage diseases, types III, IV and VI. In *Ciba Foundation Symposium Control of Glycogen Metabolism* (W. J. Whelan and M. P. Cameron eds.). Churchill, London, 1964 p. 336.
10. Johansson, S. and Ekmark, J.: Automatisk blodsockerbestämning med användande av ett färdigberett glykosoxidasreagens. *Svensk Läkartidn, 59* 3022, 1962.
11. Karlberg, P. and Ingmon, S.: A Swedish growth chart. *Acta Paediat Scand, Suppl 117* 137 1959.
12. Lamy, M., Dubois, R., Rossier, A., Frézal, J., Loeb, H. and Blancher, G.: La glycogénose par déficience en phosphorylase hépatique. *Arch Franç Pédiat, 17* 1 1960.
13. Öckerman, P. A. Glucose-6-phosphatase in human jejunal mucosa. Lack of activity in glycogenosis. I Cori's type 1. *Clin Chim Acta, 9* 151 1964.
14. — Glucose, glycerol and free fatty acids in glycogen storage disease type I. Blood levels in the fasting and non-fasting state. Effect of glucose and adrenalin administration. *Clin Chim Acta* (in press).
15. — Glycogen storage disease in Sweden. *Acta Paediat Scand, Suppl 160* 1965.
16. Sidbury, J. B., Cornblath, M., Fisher, J. and House, E.: Glycogen in erythrocytes of patients with glycogen storage disease. *Pediatrics 27* 103, 1961.
17. Williams, H. E. and Field, J. B. Low leucocyte phosphorylase in hepatic phosphorylase-deficient glycogen storage disease. *J Clin Invest, 40* 1841 1961.
18. — Further studies on leucocyte phosphorylase in glycogen storage disease. *Metabolism, 12* 464 1963.

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The Circulatory and Respiratory Adaptation to Early and Late Cord Clamping in Newborn Infants

by WILLIAM OH, JOHN LIND and IRA H GESSNER[1]

Introduction

When clamping of the umbilical cord is delayed at birth allowing placental transfusion to occur the infant receives an estimated 30 to 100 ml of blood from the placenta [13 21]. Recently Usher Shephard & Lind have shown that this blood transfusion could be as much as 61 % of the infant s existing blood volume [*4] It is likely that the rapid transfusion of this large amount of blood would result in measurable physiologic and hemodynamic alterations in the newborn infant particularly during the first few hours of post natal life This report presents serial quantitative changes in systemic blood pressures, pulse rates, and respiratory rates during the first five days of life in relation to early and late clamping of the umbilical cord. Observations on the onset of first breath and first cry at birth related to timing of cord clamping are also presented.

Recent publications have suggested a possible relationship between early cord clamping and the occurrence of respiratory distress syndrome especially in low birth weight infants [5 9 14] This has prompted renewed interest in more detailed investigations of physiologic adjustments achieved by newborn infants in response to placental transfusion.

Material and Methods

Sixty two full term newborn infants born at the Southern Maternity Hospit l, Stockholm (Sodra Barnbördshuset) were studied. These infants were products of 38 to 42 weeks of uncomplicated pregnancies, labor and delivery The verage duration of labor was 8 hours 42 min (range: 2 hours and 50 min to 26 hours and 30 min) during which time 50 of the mothers received no analgesia or anesthesia, while 12 mothers received short duration of intermittent nitrous oxide inhalation. All infants were delivered vaginally with cephalic presentation and without the aid of forceps pplication. They were delivered into the bed on which the mothers were lying, and were about 10 cm below the position of the introitus. There were 36 female and 26 male infants, with an average birth weight of 3410 g The infants were divided into two groups according t the manner in which the umbilical cords were

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clamped after birth (delivery of the buttocks was considered as the time of birth)

Group I Early clamped group (3* infants) The cords were clamped with forceps as soon as the infant s buttocks were delivered. The mean time of clamping was 7 6 sec after birth (range: 1 to 36 sec). Cords of 23 infants were lamped less than 5 sec, 5 t 10 sec 2 at 15 sec and at 36 sec after birth.

Group II Lat clamped group (30 in fants) The cords were tied and severed after the umbilical arterial pulsations ceased. The mean time f cord lamping was 3 min and 54 sec after birth. (Range 2 min 30 sec to 5 min.) No attempt was made to milk the cord before clamping.

During the delivery one observer timed the moment of infant s birth with a stop-watch and a second observer marked the time of the first breath (the first inspiratory gasp or effort taken by the infant) and first cry with another stopwatch. At 5 min of ge the Apgar score [1] was made Soon after birth the infants were transferred t the nursery where the room temperature was maint med at 23° to 25°C All infants were covered with a cotton sheet and wool blanket and at about one hour of age they were gi en warm water washing weighed, and then lothed with a cotton infant shirt, per and covered again with the cotton sheet and blanket All infants were breast fed at 12 hours of age

In 25 nfants from the early clamped group and 23 infants from the late clamped group, systolic blood pressure, pulse rates and respiratory rates were measured at 5, *10 15, 30 45 and 60 min of age and then* hourly for the first 6 hours. These deter minations were repeated t 24, 48 72 96 and 120 hours of age The systolic blood pressure was measured by the 2-cuff xylol indicator with one-inch cuff technique de scribed by Ashworth & N lgan [2] w th slight modif cation [7] All determinations were done 5 times in succession on the ext end ed right upper extremity with the infant in supine position and kept in a quiet stat Pulse rates was determined by directly counting the movement of the xylol columns on the sphygmomanometer Respiratory rates were determined by visually counting the movement of the chest and abdomen. Both pulse and respiratory rates were counted for a full minut repeated by another observer and the average obtained.

Venous hematocrit was measured at 1 and 4 hours of age and again at 24 hours to 3 days of life. The hematocrits were measured *in triplicate on scalp venous blood* samples, by microtechnique [12] using pre heparinized capillary tubes and centrifuged at 11 000 rpm for 4 min, the packed red cell column was read on a Hematocrit Read ing Chart (A. H Thomas Co Philadelphia)

To compare the -cuff xylol indicator tech nique of measuring systolic blood pressure with that of direct arterial blood pressure reading, aortic pressures were directly mea-ured simultaneously with the 2-cuff xylol indicator method on 16 newborn infants of various age groups. A number 5 polyethylen feeding tube was inserted under strict asep-tic technique into the umbilical artery to about 10–12 cm depth from the abdominal wall The arterial pressure was measured by an Elema strain gauge with the mid-chest level taken as the zero reference point and the pressure recorded on a direct writing 4 hannel Elema Mingograph. While the pressure curve was being recorded contin ously on the Mingograph one of us simul taneously made fi e successive determina-tions of systolic blood pressure by the xylol indicator method. The simultaneous deter minations were repeated three times on each infant during one xperimental procedure The mean differences between the two tech niques and their standard deviations and correlation coefficient were calculated (Table 1)

Results

As shown in Table * there was an even distribution of sex duration of labor and birth weight in the 2 groups of infants. The immediate neonatal conditions of the infants were normal as shown by the Apgar score at 5 min of age

TABLE 1 *Comparison between systolic aortic pressure directly measured and systolic arter pressure obtained by 2-cuff xylol indicator method in 16 normal newborn infants*

Number of infants	Number of deter-minations	Age (hours)	Systolic aortic pressure (mm Hg)	Systolic blood pressure by 2-cuff method (mm Hg)	Differences between aortic and 2-cuff pressure	Correlati coefficie ( )
16	196	Mean 3.2 range ½ to 8	Mean 68 S.D. ±6.8	Mean 72 S.D ±7.4	Mean +4.0 S.D ±4.1	0.83

Of the 32 early clamped infants the time of the first breath was recorded in 29 Twenty two infants breathed after the cords were clamped. The mean time of cord clamping for this group was 4 0 sec, first breath was 6.3 sec with standard error of the mean of 0 60 sec. The late clamped infants took their first breath at 9.2 sec with standard error of the mea of 1.3 sec. A statistically significant d ference was present ($p < .05$) There w no difference in the time of first cry

The late clamped infants had a high systolic blood pressure at birth than the infants whose cords were clamped immediately (Fig. 1). The systolic blo

TABLE *Sex, labor birth weight Apgar score and t me of cord clamping 1st bre and cry of 62 infants with early and late cord clamping*

		Early clamped group	Late clamped group
No. of infants		32	30
Male		14	12
Female		18	18
Duration of labor	m	7 hr 10 min	10 hr 18 min
	range	4 hr to 26 hr 20 mi	2 hr 30 min t 24 hr 30 min
Birth weight	m	3340 g	3450 g
	range	2640 to 4000 g	2730 to 4600 g
Apgar 5 min	m	9.5	9.3
	range	8 to 10	7 t 10
Time cord was clamped	m	7 6 sec	3 min 54 sec
	range	1 t 26 sec	2 min 30 sec to 5 min
Time of 1st breath	m	6 3 sec[a]	9.2 sec
	S.E.M.	±0 60 sec	±1.3 sec
Time of 1st cry	m	13 5 sec	15.0 sec
	S.E.M.	±2.5 sec	4.8 sec

[a] Of 29 early clamped infants on whom initiation of 1st breath was timed, 22 breathed after the cords were clamped. Mean time of cord clamping for this group was 4 sec and first breath, 6.3 sec as indicated in the table Test for the difference in the time of first breath between the 22 early clamped infants and the 20 late clamped infants was significant ($p < .05$).

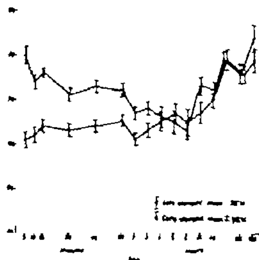


Fig. 1 Systolic blood pressure of .5 late clamped and 25 early clamped infants during the first 5 days of lif



Fig. 2. Respiratory rates of 25 lat clamped and 25 early clamped infants during the first 5 days of life.

pressure values in the late clamped infants declined from an average of 80 mm Hg at 5 minutes of age to 62 mm Hg at 6 hours of age It rose to 73 mm Hg at 24 hours and continued to rise and reached 84 mm at the 5th day of life In the early mped group of infants the systolic blood pressure at birth was lower than the late clamped infants (61 ± 1.8 mm Hg). These infants demonstrated a steady systolic blood pressure during the first 24 hours of life followed by a rise to 70 mm Hg at 48 hours of age and 79 mm Hg at the age of 5 days. It should be noted that the late clamped group of infants had an earlier rise of systolic blood pressure at 4 hours of age so that the difference in the blood pressure between the two groups at this age is also statistically significant (p = < .05) (Table 3 and Fig 1)

Both groups of infant had respiratory rates of 51 to 60 per min during the first hour of life (Table 3) However in contrast to the early clamped infants whose respiratory rate decreased to 48, 43 and 43 times per min at 1 2 and 3 hours of life the late clamped group of infants continued to breathe at 60 5_ and 51 times per min at 1 2 and 3 hours of life respectively The differences in respective rates at these ages between the two groups were significant (p = < 05) Thereafter the respiratory rates of the two groups of infants were similar (Table 3 and Fig _)

In Fig 3 the pulse rates of the early and late clamped infants during the first 5 days of life were presented Both groups of infants had pulse rates of 148 to 188 per min at birth, followed by a gradual fall to a low of 100 to 106 times per min at 6 hours of age The fall seems to occur at a steady rate during the first 6 hours of life This was followed by a gradual rise over the next 5 days of life There wa no difference in the pulse rates between the groups.

TABLE 3. *Systolic blood pressures and respiratory rates of 25 early clamped and 25 late clamped group of infants during the first 5 days of life*

Values represented were the means ± standard error of the means. N = number of infants and asterik signs on the *t* values indicate statistical significance of $p < .05$.

Age		Systolic blood pressure, mm Hg: Late clamped group	Systolic blood pressure, mm Hg: Early clamped group	t	Resp rate/min: Late clamped group	Resp rate/min: Early clamped group	t
5 min	M ± S.E.M.	80 ± 1.9	61 ± 1.8	7.2*	57 ± 2.5	5_ ± 2.6	1.1
	N	22	_5		21	22	
10 min	M ± S.E.M.	74 ± 1.4	61 ± 1.6	5.6*	60 ± 2.7	51 ± 2.8	2.3
	N	18	24		17	21	
15 min	M ± S.E.M.	78 ± 1.1	64 ± 1.3	7.1	59 ± 2.9	56 ± 2.7	0.7
	N	24	24		24	23	
30 min	M ± S.E.M.	71 ± 1.6	63 ± 1.4	3.6*	55 ± 2.5	55 ± _2	0.0
	N	25	25		23	23	
45 min	M ± S.E.M.	73 ± 1.4	67 ± 1.3	4.7*	58 ± 2.9	52 ± 1.9	1.7
	N	22	24		21	22	
1 hr	M ± S.E.M.	72 ± 1.5	65 ± 1.2	3.7*	59 ± 3.0	48 ± 1.76	2.6
	N	23	25		21	25	
2 hr	M ± S.E.M.	67 ± 1.2	61 ± 1.3	3.2*	5_ ± 1.5	43 ± _0	3.5*
	N	22	22		22	21	
3 hr	M ± S.E.M.	68 ± 1.7	63 ± 1.5	2.1	51 ± _4	43 ± 1.9	2.5
	N	21	17		20	19	
4 hr	M ± S.E.M.	66 ± 2.5	65 ± 2_	0.3	44 ± 1.7	41 ± 2.1	1.0
	N	18	17		17	17	
5 hr	M ± S.E.M.	65 ± 2.6	67 ± 2.4	0.5	44 ± 2.0	40 ± 2.2	1.2
	N	14	11		1	10	
6 hr	M ± S.E.M.	63 ± 1.9	65 ± 3.4	0.5	49 ± 2.1	40 ± 3.1	2.9
	N	8	9		9	9	
24 hr	M ± S.E.M.	73 ± 2.0	67 ± 1.2	2.5*	47 ± 1.8	44 ± 2.1	1.0
	N	24	25		24	24	
48 hr	M ± S.E.M.	72 ± 2.0	70 ± 1.8	0.7	45 ± 1.7	44 ± 1.5	0.4
	N	16	22		18	22	
72 hr	M ± S.E.M.	79 ± 2.9	79 ± 2.1	0	41 ± 2.1	45 ± 1.6	1.4
	N	19	18		18	19	
96 hr	M ± S.E.M.	78 ± 2.2	78 ± 2.1	0	43 ± 2.1	43 ± 2.1	0
	N	18	19		17	18	
120 hr	M ± S.E.M.	84 _ 3.1	79 ± 2.8	1.2	41 ± 1.6	46 ± 1.7	2.0
	N	13	15		11	15	

In 61 infants in whom venous hematocrits were obtained during the first 4 hours of life there was a direct correlation between the hematocrit and the simultaneously measured systolic blood pressure. This correlation was highly significant ($p = .01$–$.001$) (Fig 4)

In 51 infants over 24 hours of age there

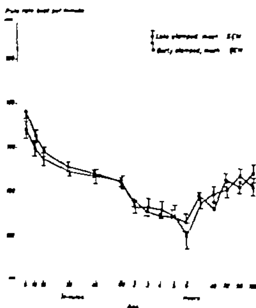


Fig. 3. Pulse rates of 25 infants from the late clamped group and 25 infants from the early clamped group during the first 5 days of life.

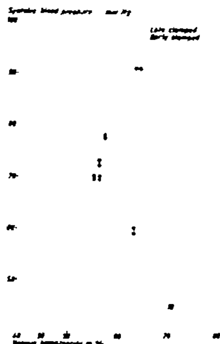


Fig. 5. Correlation between venous hematocrits (x) and systolic blood pressure (y) in 50 infant over 24 hours of age

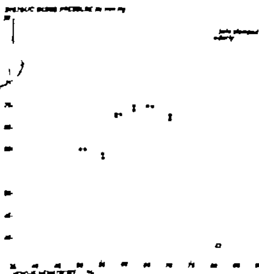


Fig. 4. Correlation between venous hematocrit (x) and systolic blood pressure (y) in 50 new born infant with 6 simultaneous determinations during first 4 hours of life (p = 01 001).

was no correlation between the venous hematocrits and simultaneously measured systolic blood pressures (Fig. 5)

Discussion

Chemical sensory and thermal stimulations play a major role in the initiation of respiration at birth [3]. High CO_2 and low O_2 concentrations in the blood are capable of stimulating the respiration center situated in the carotid sinus and medulla [10]. The earlier onset of respiration in the early clamped infants is most likely due to an earlier fall in blood oxygen saturation and elevation of carbon dioxide contents resulting from the earlier interruption of placental circulation.

The systolic blood pressure in the new born infant has been the subject of several

investigations [–, 6, 7 15 19 20 26 27]. In most of these reports the measurements were not performed serially nor at frequent intervals, and only two reports attempted to relate blood pressure to early and delayed umbilical cord clamping [–, 8].

Ashworth & Neligan [2] measured the blood pressure changes almost immediately after birth and demonstrated a decline in systolic blood pressure during the first 24 hours of life. They have also shown that blood pressure level of the early clamped infants declined more rapidly than the late clamped infants, although the magnitude of fall were the same. Contis & Lind also observed a fall in systolic blood pressure during the first few hours of life in forty newborn infants [7] although they did not specify the time of cord clamping. Cort & Pribylova found that infants with maximal placental transfusion as a group had systolic blood pressure some 5–6 mm Hg higher than those with small transfusion although the difference was not statistically significant [8]. Our observation of high systolic blood pressure at five min of life followed by a rapid decline during the first 6 hours and subsequent rise in the 2nd day in the late clamped infants is in conformity with the observations of other workers [2, 7]. In the early clamped group, however our results demonstrated that the systolic blood pressure starts t a much lower level than the late clamped infants and is followed by slight rise during the first 15 min of life. Thereafter it remained at a slightly lower level than the late clamped infants (T ble 3 Fig. 1). Differences in blood volumes, plasma volumes and red cell volumes constitute the basic effect of early and late cord clamping [4] It is likely that systolic blood pressure differences found between the early and late clamped infants are in large part, due to the differences in their blood volumes [4 22, 25], the positive correlation between systolic blood pressures and simultaneously determined venous hematocrits during the first 4 hours of life (Fig 4) further supports this assumption, since venous hematocrit has been shown to correlate well with blood volume [24]

Several indirect methods, such as palpation [19], flush [15] phletysmographic [6], and the present [2] methods have been employed to measure systemic blood pressure in the newborn infant. However relatively little information is available comparing indirect readings with simultaneously obtained direct intra arterial pressure [7 16]. In 16 infants in our study the 2-cuff method indicated a 4 mm higher pressure than the direct aortic reading with a correlation coefficient value of 0.83. This observation, along with the findings of Contis & Lind [7], indicates the satisfactory accuracy of the 2-cuff xylol indicator technique for measuring systolic blood pressure in neonates.

In spite of the lower systolic blood pressure and slower respiratory rate observed in this study and a lower peripheral cutaneous temperature found in a separate experiment [17] among the early clamped group of infants when compared with the late clamped infants during the first hours of life no significant difference in the pulse rate was detected between the two groups. This finding is both perplexing and interesting

In our study both early and late clamped group of infants showed respiratory rates of 51 to 60/min, during the first half hour

of life. However from one hour of age to three, the respiratory rates of the early clamped infants declined to a lower level, while in the late clamped group of infants it remained at a higher level. The differences in respiratory rates between the 2 groups during one to three hours of age being statistically significant (Table 3 Fig 2) It is of interest that the difference in respiratory performances between the early and late clamped infants occurred at the age when capillary fluid transudation is presumably at its maximal degree as shown by the simultaneous venous and capillary hematocrit measurement in the late clamped infants [18] It was suggested that infants given placental transfusion at birth adjust to the vascular distension by a process of active fluid transudation [11 23 24] occurring at the capillary bed [18] Although quite highly hypothetical it is possible that such process may occur in the pulmonary capillary bed resulting in a mild degree of pulmonary edema and thus a faster respiratory rate. It is evident that more detailed study of the pulmonary chanics and functions is necessary to clarify this observation.

Summary

Time of first breath and frist cry systolic blood pressure pulse rate and respiratory rate of 32 newborn infants whose umbilical cords were clamped early and 30 whose cords were tied late after birth were studied The early clamped infants breathed significantly sooner after birth than the late clamped infants, probably a result of anoxia due to severance of placental blood supply by immediate cord clamping The indirectly measured systolic blood pressure was significantly higher in the late clamped group of infants during the first 24 hours of life perhaps due to the difference in blood volumes between the two groups. This is further supported by a positive correlation found between systolic blood pressure and venous hematocrit during the first 4 hours of life Pulse rate in the two groups of infants studied revealed no significant difference. The respiratory rate in the early clamped group of infants was significantly slower during the 1st to the 3rd hour of life when compared with the late clamped infants. No satisfactory explanation could be offered for this observation.

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References

1 Apgar, V A proposal for a new method of evaluation of the newborn infant. *Anesth Analg (Cleveland)* 32 260, 1953
2. Ashworth, A. M. and Neligan, G A.: Changes in the systolic blood pressure of normal babies during the first twenty-four hours of life *Lancet, 1* 804, 1959
3. Avery M. E.: *The Lung and Its Disorders in the Newborn Infant* W B Saunders Co. Philadelphia, 1964
4 Best C. H. and Taylor, N B.: *The Physiological Basis of Medical Practice* p. 280 7th Ed. Williams and Wilkins, Baltimore 1961
5. Bound J P Harvey P W and Bosher H. B.: Prevention of the pulmonary syndrome of the newborn. *Lancet, 1* 1200, 1962
6 Celander, O and Tunnell, G A plethysmographic method for measuring the systolic and diastolic blood pressure in newborn infants. *Acta Paediat Scand, 49* 497 1960.

7 Cottis, G and Lind J Study of systolic blood pressure, heart rate, body temperature of normal newborn infants through the first week l lif Acta Pædiat Scand, 146 41, 1963.

8. Cort R. L. and Pribylova, H.: Placental transfusion and fluid metabolism on the first day of life. Arch Dis Child 39 363, 1964

9 Dorms, H. A., Franklin R. R., Hensley W S., and Desmond M. M.: Blood volume in respiratory distress in the neonate.—A preliminary report. Circulation, 24 920, 1961 (abst.).

10. Eastman N J Geiling E. M. K. and Delawder, A. M.: Fetal blood studies: Oxygen and carbon dioxide dissociation curve of fetal blood. Bull Johns Hopkins Hosp, 53. 246, 1933.

11 Gairdner, D Marks, J Roscoe, J D and Brettell, R. O The fluid shift from the vascular compartment immediately after birth. Arch Dis Child, 33 489, 1958.

12. Guest G M. and Silver, V G Centrifuge method for determination of volume of cells in blood. J Lab Clin Med, 19 757 1934.

13. Gunther, M.: The transfer of blood between the baby and placenta in the baby and placenta in the minutes after birth. Lancet 1 1277 1957

14. Moss, A. J Duffie, E. R. and Fagan, L. M.: Respiratory distress syndrome in the newborn. JAMA, 184 48, 1963.

15. Moss, A. J Liebling, W and Adams, F H.: The flush method for determining blood pressure in infants. II. Normal values during the first year of life. Pediatrics 21 950, 1958.

16. Moss, A. J and Ad m F H.: Flush blood pressure and intraarterial pressure. Amer J Dis Child, 107 489 1964

17 Oh, W and Lind J Regulation of body temperatures in newborn infants with early and late cord clamping I manuscript.

18. Oh, W and Lind, J V nous and capillary hemotocrit in newborn nfants and placental transfusion. Acta Pædiat Scand, 55 38, 1966.

19 Ruelbke, M. P and Conell J W Blood pressure in the newborn. Amer J Dis Child 47 6, 1934.

20 Schaffer, A.: Neonatal blood pressure studies. Amer J Dis Child, 89 204, 1955.

21 Secher, O and Karlberg P Placental blood transfusion. Lancet 1 1203, 1962.

22. Shenkin H. A., Cheney R. H., Govons S. R., Hardy L D Fletcher, A G and Starr, I.: On the diagnosis of hemorrhage in man. A study of volunteers bled large amounts. Amer J Med Sci, 208 421 1944.

23. Steele, M. W Plasma volume changes in the neonate. Amer J Dis Child, 103 10, 1962.

24. Usher, R., Shephard M. and Lind J The blood volume of the newborn infant and placental transfusion. Acta Pædiat Scand, 52 497 1963.

25. Wallgren, G Barr, M. and Rudhe, U Hemodynamic studies of induced acute hypo- and hypervolemia in the newborn infant. Acta Pædiat Scand, 53. 1 1964.

26. Woodbury R. A., Robinow M. and Hamilton W F Blood pressure studies on infant. Amer J Physiol, 122 472, 1938.

27 Young, M.: Blood pressure in the newborn baby Brit Med Bull, 17 154, 1961

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Nitrogen and Fat Retention in Premature Infants Fed Breast Milk, ' Humanized Cow s Milk or Half Skimmed Cow s Milk

by J ZOULA, V MELICHAR, M. NOVAK, P HAHN and O KOLDOVSKY

The optimum system of feeding new born and particularly premature infants is still a matter of discussion. In Czechoslovakia breast milk has always been regarded as the best food for premature infants, but some authors prefer feeding formula milk instead of breast milk, since the latter is thought to contain too little protein and too much fat [1 8 9 14 15]

When breast milk is not available half skimmed cow s milk which contains considerably more protein and minerals and less fat is largely used in this country [24 _5]. In recent years so called humanized milk has been introduced in some countries the composition of which approaches breast milk more closely

The higher content of fat of such milk may be of particular interest since the importance of fat during the suckling period in both man and animals has been demonstrated [10 11 18 19].

In the present paper an attempt is made to compare by balance studies the retention of nitrogen and fat in premature infants fed. (1) breast milk, ( ) humanized" milk and (3) half skimmed cow s milk.

Subjects, Procedures and Methods

Balance studies with different diets can be performed in 2 ways: (1) by comparing different infants of the same age fed different diets or (2) by feeding the same infant different diets in successive time intervals. The second method was chosen for several reasons: *First*, interindividual differences are eliminated and only age differences can interfere *Second* it is ery difficult to determine the exact degree of immaturity and in the second method each infant is its own control. *Third* previous experiments [28] have shown that between the 3rd and 8th week fter birth no changes in nitrogen retention occur in immature infants (retention/int ke in the 3rd week—on breast milk = 0.62 s.e. ± 0 045; retention/intake in the 8th week,—on breast milk = 0.58 s.e. ± 0 064 retention/intake in the 3rd week—on half skimmed milk 0.40 s.e. ± 0.040; retention/intake in the 8th week—on half-skimmed milk = 0.46 s.e. ± 0.036) The work of Fomon [7] also shows that no significant changes in nitrogen retention are seen between the 20th and 60th day of lif in full term infants. The mean age of our infants when fed the diet was 34 33 and 40 days for breast milk "humanized" milk and skimmed milk, respect vely

Balance studies were performed with 12

TABLE I *Characteristics of 12 immature infants order of feeding b lance studies*
Breast milk—B, "humanised" milk—H, half skimmed milk (Eviko, Lakton)—1/²E, L.

No.	Subject	Birth wt.g/ length cm	Gestation age in weeks	Order of feeding	Age at start and end of study in days	Weight at start and end of experiment in g	Remarks
1	D A.	1800/43	33	B H 1/2 E	33–61	2080–3110	Clinically normal
2	D B.	1690/4_	33	B H 1/2 L	33–61	1870–2930	Breech presentation, clinically normal
3	H. R.	1400/37.5	31	B 1/2 E H 1/2 L	26–63	1610–2680	Slight hypochr anemia t end of study
4	H. L.	2005/44	32	H 1/2 L H	17–46	2190–3100	Breech presentation, clinically normal
5	H. U	1780/42	32	B H 1/2 L	36–60	1950–2720	Slower recovery f birth weight
6	K. L.	1800/42	34	H 1/2 E B	25–52	2200–2930	Clinically normal
7	L. O	2200/45	35	H 1/2 L H	18–44	2190–*890	Clinically normal
8	B. U	2070/43	34	H 1/2 E B	17–40	1890–2400	Aspiration at birth, large initial fall in body weight
9	P O	2100/46	36	H 1/2 L H	*0–44	2030–*900	Breech presentation, extraction
10	A. D	2000/45	33	H 1/2 L	23–37	1970–2400	Large initial fall in body weight
11	S. O	2400/47	36	H 1/2 E	15–27	2460–2830	Clinically normal
12	D O	2170/45	35	B H 1/2 L	15–40	2210–3000	Clinically normal

immature male infants. Table 1 gi es their characteristics.

Two t three types of diet were tested in each infant following an adaptive period of 5–14 days. The three types f milk used are shown in Table 2, which gives values deter mined in the laboratory

Each balance experiment lasted for at least 4 days. A total of 35 such experiments was performed with the 12 infants (140 experimental days) Seven experiments were performed with breast milk, 13 with half skimmed cow milk (5 Eviko 8 Lakton) and 15 with "humanized milk. The order in which the different diets were fed is shown in T ble 1

Urine and stools were collected in the usual way with some modifications [7 12, 27 28]. Vomiting occurred rarely and if it did, the amount of milk vomited was also included in the calculations.

Milk for one whole experiment was always prepared beforehand and stored t −20°C. The content f nitrogen and fat in all milks was always determined. Nitrogen was deter mined in milk, urine and stools using the usual Kjeldahl method, fat in the stools and milk by the method of Homolka [13], which is based on saponification of the fat with N OH subsequent a treatment of fatty acids mt petrolether Palmitic acid was used as standard.

The following basic data were then used, total nitrogen content in food. F; nitrogen content in urine: U nitrogen content in stools: St; nitrogen absorbed from intestine: A (calculated from difference between F and St), nitrogen retained in body: R (cal culated from difference between A and U).

In similar way dat for fat were calcu lated.

From the above values the following coef

TABLE 2. *The composition of the three types of diets (g/100 ml milk)*

(1) "Humanized" milk was prepared specially by the Czechoslovak Milk Industry Prague Protein and carbohydrate were the same as in cow milk, *while 4/7 of the fat were exchanged* for soya bean oil. The mineral content was decreased to nearly half that for cow milk, but the ratio of individual minerals remained unchanged.

(2) Half skimmed milk—Eviko and Lakton (produced by Czechoslovak Milk Industry Prague), re the usual artificial milk mixtures used in Czechoslovakia for infants up t the age of 6–10 weeks. They are prepared from cow s milk but contain less fat. Lakton is dried butter milk, while Eviko is dried cow's milk. T both types 5 g saccharose is added t 100 ml.

(3) Breast milk was supplied from a milk bank and was lyophilized by Léčiva n.p., Prague (Drug Industry) so that samples of stable composition could easily be prepared

Determination of nitrogen and fat as described unde method. Data on carbohydrates were obtained from the manufacturers. The usual daily doses of vitamins A, D C and B were added to all types of milk.

	Fats g/100 ml	Proteins g/100 ml	Carbo-hydrat g/100 ml	Minerals g/100 ml	Calories per 100 ml from fat and carbohydrate
(1) "*Humanized milk*	3.17	1 66	6.7	0.39	56.52
(2) Half skimmed milk					
(Lakton, Eviko)	1.08	2.54	4 0 + 5.0 (L)	0.65	48.72
			4.5 + 5.0 (E)		48.72
(3) Breast milk	3.30	1.54	7.0	0.23	58.96

ficients were obtained. F/W—the amount of fat (nitrogen) fed per kg body weight A/W—the amount f nitrogen absorbed per kg body weight R/W—the amount of fat (nitrogen) retained per kg body weight For fat $A/W = R/W$ since hardly any fat is eli minated via the kidneys.

Further nitrogen (fat) retention was rela ted to the amount of nitrogen in the milk and nitrogen retention also to the amount of nitrogen absorbed from the gut

All values were compared for the three kinds f milk nd differences were evaluated statistically using Student s *t* test.

Results

The growth of all infants was within normal limits as can also be seen from their weights at the start and end of the experiment (Table 1) The daily weight gain during the 4-day balance periods in % of the initial weight was 0.93 %, for breast milk, 1.01 % for humanized milk and 1.25 % for half skimmed milk.

The daily intake of calories was 178 ml/kg "humanized" milk = 110.30 Cal/kg 186 ml/kg skimmed milk = 108.0 resp. 103 4 Cal/kg (E resp. L)· 188 ml/kg breast milk = 120 4 Cal/kg

Table 3 and Fig 1 summarize the results.

It is evident from Table 3 that the same amount of nitrogen was absorbed from the gut on the three types of milk (A/F coefficient) Further about the same amount of nitrogen was retained in the body regardless of how much nitrogen had been fed or absorbed (Fig 1) The dif ference in nitrogen retained per unit weight between infants fed breast milk and those fed humanized" milk was significant for $p < 0.02$, the difference between half skimmed milk and humanized milk is significant only for $p = 0.05$ (Table 3)

In other words infants fed the half skimmed milk eliminated about twice the amount of nitrogen in the urine in com parison to infants fed humanized or

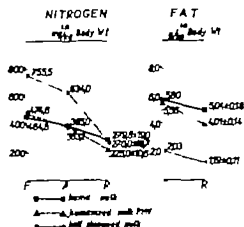


Fig. 1. Utilisation of proteins and fats in premature infants on 3 types of milk diet. F = nitrogen (fat) content in food; A = nitrogen absorbed from intestine; R = nitrogen (fat) retained in body

breast milk. Thus the coefficients R/F and R/A for nitrogen are highest for breast milk and lowest for artificial half skimmed milk (Table 3).

Fat retention increases with increasing fat supply in the food (Table 3) but in addition, the R/F coefficient signifying the amount of fat retained in the body in relation to the amount of fat in the milk, is highest for breast milk and lowest for half skimmed milk, "humanized" milk lying between the two.

Discussion

Breast milk proteins were well retained and nitrogen losses were small. The same applied for fat. The high lipid content of breast milk and "humanized" milk was well tolerated and fat was well utilized. Half skimmed artificial milk was shown to be much less favourable. Even though nitrogen absorption was extensive, relative nitrogen retention was small and fat even though less was offered than in breast

TABLE 3. *Summary of the results of balance experiments with the three milk diets*

For abbreviation see: method.

Statistical significance for nitrogen indicators
(1) for R/W I.II $p < 0.02$, II.III $p = 0.05$, I.III not sign.,
(2) for R/F I.II $p < 0.001$, II.III $p < 0.001$, I.III $p < 0.001$
(3) for R/A I.II $p < 0.001$, II.III $p < 0.001$, I.III $p < 0.001$

Statistical significance to fat indicators
(1) for R/W I.II $p < 0.001$, II.III $p < 0.001$, I.III $p < 0.001$,
(2) for R/F I.II $p < 0.001$, II.III $p < 0.02$, I.III $p < 0.001$

Mean standard error	Nitrogen in mg							Fat in mg		
	F/W	*A/W*	*U/W*	*R/W*	*A/F*	*R/F*	*R/A*	*F/W*	*R/W*	*R/F*
I *Breast milk*										
x	464.8	385.0	104..	279.8	0.82	0.60	0.72	5.60	5.05	0.96
± s. x.				19.0		0.025	0.19		0.1 6	0.021
II *"Humanized" milk*										
x	474.6	383.2	166.4	223.0	0.81	0.46	0.58	5.46	4.01	0.70
± x				10.63		0.019	0.007		0.143	0.026
III *Half skimmed milk*										
x	755.8	634.0	362.7	270.6	0.83	0.36	0.43	2.03	1.19	0.59
± x.				16.7		0.014	0.005		0.113	0.035

Nitrogen/fat in F = food, U = urine R = retained, A = absorbed, W = body weight.

milk, was retained to a lesser extent In this connection it would be useful to determine the degree of endogenous fat loss, which might be the same regardless of the amount of fat fed and might lead to an error in our calculations

Humanized milk was found to be more suitable than artificial milk with higher protein content, as has been reported previously mainly for mature infants [*-5 17 20 26]

Taking the caloric value of carbohydrate as 4 and that of fat as 9 Cal/g it is evident from Table 2 that breast milk contains 58.96 "humanized" milk 55.53 and half skimmed milk 45 72 L 48.72 E Cal/100 ml derived from carbohydrate and fat. Evidently the infant will have to derive calories from protein in increasing amounts as the number of calories supplied from fat and carbohydrate is decreased and this is shown to be so in Table 4 where the amount of nitrogen lost is also calculated in calories and is taken to indicate energy derived from protein. If we add the calories in Table 4 to give us a rough te of the total number of calories needed per unit weight and unit time by the infant we obtain the figures shown in the last row

The above three figures lie fairly close to each other and it may be assumed that the large loss of protein in infants fed half skimmed milk is due to the fact that an insufficient number of calories in the form of either fat or carbohydrate is being supplied Our experiments also indicate that evidently fat spares proteins equally well as does carbohydrate, as has been suggested previously

Since the number of calories supplied by carbohydrate is 66 for half skimmed milk against 47 or 52 for "humanized" and breast milk, while the difference for calories from fat is much higher (10 36 45 Cal) it appears that fat serves at least equally well as a source of energy as carbohydrate

It must also be borne in mind that the high protein content of half skimmed milk might increase the load on the kidneys, which are forced to deal with a much greater amount of nitrogen than in the case of breast milk and "humanized milk [4 *1].

It is noteworthy that with increasing amount of fat in the milk the percentage retained in the body also increases. Th does not always seem to be the case [22] and here many factors may play a role In any case in our experiments the more fat was retained in the body the less nitrogen was excreted in the urine again indicating though not demonstrating the sparing effect of fat.

TABLE 4 *Calories available as energy in premature infants fed three types of milk.*

Figures were obtained as follows ( ) Carbohyd rates consumed per day and kg were assumed to be fully absorbed and completely utilized. 1 g = 4 Cal. (b) F t absorbed from gut was assumed to be fully utilized 1 g = 9 Cal ( ) Nitrogen excreted was assumed to be utilized for energy 1 g nitrogen 6.5 = g protein, 1 g protein = 4 Cal.

	Cal/kg/day		
	"Humanized *milk*	Half skimmed" *milk*	Breast *milk*
Carbohydrat	47.5	66.6 70.22	52.6
Fat	36.09	10.71	45.36
Protein	3.92	9 06	–6
	87 61	86.37 90.09	100 76

In cases where breast feeding is impossible it seems advisable to give humanized milk instead of previously used artificial half skimmed mixtures, which even though they may give greater weight gains (probably due to water retention) place an extra load on the infant The only debatable effect of "humanized" milk in general is that it lowers the blood cholesterol level (probably because of the plant oil used [16, 23]) and we have no idea whether this is a desirable or undesirable result

Summary

Three types of milk diet were tested in balance experiments using premature infants They were breast milk, "humanized" milk and half skimmed cow's milk containing more protein and less fat than the other two milks.

It was ascertained that protein retention was about the same on all three diets, while nitrogen elimination via the urine was much higher on the half skimmed milk. Fat retention was lowest on half skimmed milk, but this may have been due to the low fat content of that milk.

References

1. Albanese, A. A. Protein and amino-acid requirements of infants. *Pediatrics, 8* 455, 1951
2. Beckmann E., Keller, W and Rehwerk, H.: Untersuchungen zur Frage der Frauenmilch-adaptierten Säuglingsernährung. *Medizinische, 33* 1466, 1959
3. Bremer, H.: Zum Problem "humanisierter" Milche *Moderne Probleme der Pädiatrie* II. Karger Verlag Basel, New York, 1957 p. 70.
4. Connelly D A., Barness, L. A., Gibson J and György P Comparison of prematures fed diets with two different levels of protein. *Pediat Int (Roma), 9* 121 1959.
5. Droese, W Stoffwechselphysiologische Untersuchungen über die Fette in der Ernährung des jungen gesunden Säuglings. *Kinderärztl Prax 29* 391, 1961
6. Fomon S. J Comparative study of adequacy of protein from human milk and cow' milk in promoting nitrogen retention by normal full-term infant. *Pediatrics, 26* 51, 1960.
7. Fomon S. J Thomas, L. E., Jensen R. L. and May C D Determination of nitrogen balance of infants less than 6 months of age. *Pediatrics, 22.* 94, 1958.
8. Gordon, H H., Levine, S. Z. and McNamara, H.: Feeding of premature infants. Comparison of human and cow's milk. *Amer J Dis Child 73* 442, 1947
9. Gordon H. H. and McNamara, H.: Fat excretion of premature infants. I. Effect on fecal fat of decreasing fat intake *Amer J Dis Child 62* 328, 1941
10. Hahn P Koldovský O Melichar, V and Novák, M.: *The Development of Homeostasis.* Symposia of the Czechoslovak Academy of Sciences. Publishing House of the Czechoslovak Academy of Sciences, Prague 1960/1961 p. 141
11. — Fat metabolism as related to carbohydrate metabolism in infants rats. BBA Library edited by Elsevier Publishing Company Amsterdam 1963, vol. I, p 258.
12. Hoag, L. A.: Apparatus for the quantitative collection of urine and of stools in male infants. *Amer J Dis Child 44* 770, 1932.
13. Homolka, J Chemical diagnostics in infants. (In Czech.), SZdN Praha, 1956, p 412.
14. Levine, S. Z.: Feeding of premature infants. *J Nutr 37* 511 1949
15. — Protein nutrition in pediatrics. *JAMA 133* 293, 1945.
16. Lindquist B. and Malmcrona R.: Dietary fat in relation to serum lipids in the normal infant. *Amer J Dis Child, 99* 39 1960
17. Löhr, H. and Wolf H.: Bilanzstudien mit fettangereicherter Milch. *Pediat Int (Roma), 9* 69, 1959
18. Melichar, V Novák, M Hahn P Koldovský O and Zeman L Changes in the blood levels of lipid metabolites and glucose following fatty meal in infants. *Acta Pediat Scand 51* 481 1962.
19. Novák, M., Melichar, V Hahn, P and Koldovský O Levels of lipids in the blood of newborn infants and the effect of glucose administration. *Physiol Bohemoslov, 10* 488, 1961.

20. OMANS, W. B., BARNESS, L. A. et al.: Prolonged feeding studies in premature infants. J Pediat 59 951 1961.
21. ROTHE MAYER, A. and SANDE, E.: High protein nutrition in prematures by means of casein supplement to human milk. Acta Paediat Scand 45. 491, 1956.
22. SMITH, C. A.: The physiology of newborn infant. In Fat Assimilation and Metabolism, III. Edit. Thomas, Springfield, 1959
23. SWEENEY M. J., ETTELDORF J. N. et al. Dietary fat and concentrations of lipid in the serum during the first to eight weeks of life Pediatrics, 27 765, 1961
24. ŠVEJCAR, J. Development of Czechoslovak system of artificial feeding of infants. (In Czech.) Čsl Pediat 13 868, 1958.
25. ŠVEJCAR, J. HNÁTEK, J. DLECHAN, J and HEČKO, L.: Feeding children. (In Czech.) Čsl Pediat, 10 161 1955.
26. TAKATSU T. The protein requirement of premature and normal infants. (In Japanese.) Paediat Uni Tokio, 6 13, 1961 Ref. Zbl. Kinderheilk, 83 44 1962.
27. TOLKAČEVSKAJA M. F. Development of Metabolism in Children during the First Year of Life (In Russian.) AMN SSSR, Moskva 1951 p. 11
28. ZOULA, J. Dissertation, Prague 1962.

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The Importance of Decalcification in the Treatment of Tuberculosis

I The Influence of Decalcification in the Course of Healing in Experimental Tuberculosis in Guinea pigs[1]

by TADEUSZ GIZA, MAGDALENA HANICKA, ADAM JELONEK, ANDRZEJ KULIG HALINA REMBIESOWA and MAREK GARAPICH

While severe and formerly invariably fatal forms of tuberculosis (miliary tuberculosis and meningitis) can now be treated successfully with tuberculostatic drugs such as streptomycin, isoniazid, etc., the so-called tertiary tuberculosis continues to present considerable therapeutic problems. The difficulties arise partly from the fact that calcified pathologic pulmonary tissue is not easily penetrated by drugs. The calcification of caseous tuberculosis foci is commonly regarded as typical dystrophic calcification, such a occurs in number of other diseases. It has been found, however that non-caseous tuberculous tissues also contain more calcium than healthy tissues [5 6 8] A significantly higher uptake of radioactive calcium by tuberculous organs in guinea pigs, proportionate t the degree of advancement of the tuberculous process has also been reported [3 4], suggesting that calcium tends to accumulate in the tissues of a body infected with tuberculosis

The purpose of the research here reported was to study the influence of calcifying and decalcifying agents on the course of the specific therapy of experimental tuberculosis in guinea pigs.

Material and Methods

The experiment was performed from November 1963 to March 1964 using 60 ten-week-old male guinea pigs weighing 310–560 g The animals were tuberculin-negative and were fed on oats, hay pasture beets and carrots. They were inoculated with the human strain Mycobacterium t berculosis $H_{37}R_v$ subcutaneously in the right inguinal region in doses of 0.1 mg of bacterial mass suspended in 0.5 ml of physiologic saline per animal.

Tuberculin tests were performed with a 1 % solution of old t berculin Koch, produced by the Serum and Vaccine Production Laboratories in Warsaw

Four weeks after inoculation all the animals exhibited positive tuberculin reactions. Therapy was begun six weeks after inoculation streptomycin in doses of 0 1 mg and isoniazid in doses of 0.2 mg per 100 g body weight of the animals.

The guinea pigs were di ided into three groups, each numbering 18 animals

The investigation was sponsored by the Polish Academy of Sciences

Freshly passaged strain received from the Institute of Tuberculosis in Warsaw

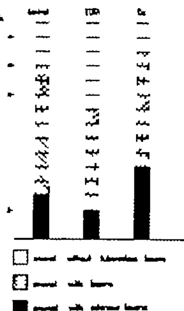


Fig 1 Distribution of the anatomopathologic changes in the different groups of animals.

Group I—controls—received only specific therapy;

Group II in addition to specific therapy were treated with trisodium versenate (EDTA Natrium) of pH 7 0 in doses of 5 mg per 100 g body weight injected intraperitoneally as a decalcifying agent;

Group III in addition to specific therapy were treated with calcium in the form of galactogluconate in doses of 0.2 mg per 100 g body weight

The influence of EDTA and of calcium on the course of tuberculosis in guinea pigs not treated specifically was studied in three subgroups of 5 animals each.

Ia—physiologic saline solution was injected subcutaneously and intraperitoneally;

IIa—EDTA Natrium was injected intraperitoneally in doses of 5 mg per 100 g body weight and physiologic saline solution subcutaneously;

IIIa—calcium in doses of 0.2 mg per 100 g body weight as galactogluconate was injected intraperitoneally and physiologic saline subcutaneously

These subgroups served as controls for the main groups; each subgroup was compared with the analogous group receiving additionally specific therapy

Twelve weeks after inoculation (7 weeks after beginning therapy) the animals were sacrificed under ether anesthesia. Each animal was studied anatomically and histologically and the calcium content of the lungs and spleens were determined.

Results

Despite lymphonodular changes at the site of injection of the bacilli and positive tuberculin tests, gross inspection failed to reveal tuberculous dissemination in the internal organs of 3 guinea pigs of the control group 6 of the decalcified group, and * of the group treated with calcium.

The extent of the visceral tuberculous lesions, scored according to Feldman, was least in the EDTA group (* animals), greater in the control group (3 animals) and greatest in the calcium group (5 animals) (Fig 1)

Sections from all the organs, stained with hematoxylin and eosin, were studied histologically The visceral changes were scored at first according to Barcikowski & Ruszczewski [1] based on the dynamics of the tuberculous process. This classification *takes into account inflammatory* lesions without signs of specificity produced by tubercle bacilli, as well a tuberculous lesions of different degrees of intensity

If the presence of tuberculous granulation tissue in the organs is regarded the criterion, specific changes were absent in 7 of the animals of the control group, 9 of the EDTA group and 6 of the calcium group.

Extensive tuberculous lesions, scored 4 and 5 according to Barcikowski & Rusz

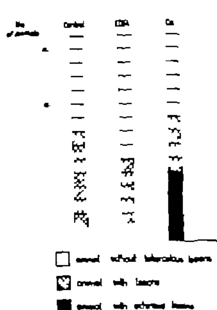


Fig. 2. Distribution of the histologic changes in the different groups of animals.

czewski, i.e. tuberculosis diffusa cum necrosi, were distributed as follows:

In the control group changes of this degree were observed in one animal in two organs, in the EDTA group in one animal in three organs, and in the calcium group in five animals in six organs (Fig). Since the evaluation of the histologic changes according to Barcikowski & Ruszczewski does not reflect the composition of the tuberculous granulation tissue the histologic analysis was made with the histologic index of Hornung et al [ ] This index takes into account the quantitative proportions of epithelioid cells, giant Langhans cells and necrotic foci. A similar composition of the tuberculous granulation tissue was found in the different groups. Hence the differences observed were only quantitative and not qualitative

The calcium content in the lungs and spleens was assayed by the method of Reynolds & Linde [7] with an Uvispek Hilger spectrophotometer Significantly lower calcium levels were found in the decalcified animals as compared with the control group On the other hand, the differences between the group of animals treated with calcium and the two remaining groups were not significant (Fig 3)

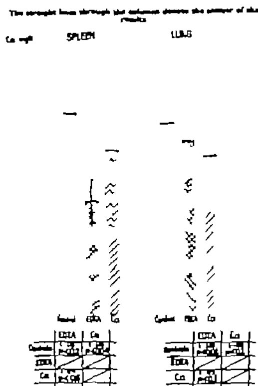


Fig. 3. Content of calcium in the spleens and lungs of animals of the different groups.

In subgroups Ia, II and IIIa all the animals exhibited marked emaciation and extensive specifi visceral lesions. The tuberculous lesions were most extensive and the loss in body weight was greatest in group IIa (EDTA N trium) animals.

The animals in the subgroups died be-

tween the 4 th and 68th days after infection.

On comparing groups I II and III with the corresponding subgroups Ia IIa and IIIa the greatest disproportions in the anatomico- and histopathologic changes were observed between group II and subgroup IIa, suggesting that EDTA Natrium affects the course of experimental tuberculosis adversely but enhances the effect of specific therapy

Discussion

Preliminary observations on the action of EDTA on healthy guinea pigs disclosed a certain degree of toxicity of the drug manifested by loss of appetite poor gain in weight and restlessness after administration of the drug, in spite of absence of gross and microscopic visceral lesions.

It may be concluded that in spite of toxicity the combined administration of EDTA Natrium and tuberculostatic drugs enhances the therapeutic effect.

The findings in this study indicating a favorable effect of decalcification on the course of the specific therapy of experimental tuberculosis point to the possibility of a new trend in the therapeutic management of this socially important disease after suitable clinical trials.

Summary

The authors have the opinion that by percalcification of the morbid pulmonary tissue in destructive pulmonary tuberculosis impedes the requisite penetration by the therapeutic agents employed. Experiments were made on guinea pigs, one group receiving antibiotics together with chemical compounds of the EDTA type having a decalcifying effect, while a second group received calcifying agents as well as therapeutic drugs. For control, a third group was treated solely with streptomycin and isoniazid. Chemical anatomicopathological and histopathological tests provided criteria for evaluation. The best therapeutic results were obtained in the group of decalcified animals.

References

1 Barcikowski, W and Rubczewski, Z.: A proposed method of graphical presentation of the morphological lesions in experimental tuberculosis (Próba graficznego ujęcia zmian morfologicznych w gruźlicy doświadczalnej). *Patologia Polska, 11* 249, 1960.

2 Horodko, S Kowalczykowa, J Parynski E., Kulig A., Latak, Z., Polonczyk, M and Wasowicz, A.: The course and morphological appearance of experimental tuberculosis in guinea pigs infected with strains of Mycobacterium tuberculosis isolated from INH-treated tuberculous patients (Przebieg i obraz morfologiczny gruźlicy doświadczalnej świnek morskich wywołanej przez szczepy Mycobacterium tuberculosis wyosobnione od chorych leczonych INH). *Gruźlica, 27* 6?1 1959

3 Ido, H Studies on calcium metabolism in experimental tuberculosis. *K Hal* 36 14.., 1961 (English abstract.)

4 Jelonek, A.: Ca^{45} uptake by organs of tuberculous guinea pigs (Wychwyt Ca^{45} przez gruźlicze zmienione narządy świnek morskich). (In press.)

5 Löwy A. and Gutzeit, H Über die Wirkung längerer Vigantol-Calciumzufuhr auf gesunde und tuberkulöse Tiere *Z Tuberkul 61* 317 1931

6. PLEBAŃCZYK, P Vitamin D and cod-liver oil in therapy of tuberculosis (Witamina D i tran w leczeniu gruźlicy). *Gruźlica, 10* 491, 1935.

7 REYNOLDS, E. S. and LYNDE, R. E.: Colorimetric determination with Murexid of microgram amounts of calcium in tissue *Analyt Biochem, 5* 246, 1963.

8. SPIES, T and WALKER, T A comparison of the effects of viosterol in acute experimental avian and bovine tuberculosis. *Amer Rev Tubercul, 24* 722, 1931

9 ZEYLAND, J Administration of calcium in tuberculosis (O stosowaniu wapnia w gruźlicy). *Polski Tygodnik Lekarski, 2.* 41 1946.

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Venous and Capillary Hematocrit in Newborn Infants and Placental Transfusion

by WILLIAM OH[1] and JOHN LIND

It has previously been shown that in the newborn infants during the first week of life the hemoglobin and red blood cell counts of the capillary blood are significantly higher than their simultaneously obtained venous values [3, 12] in contrast to that of adults where the capillary hematocrits (packed red cells) are slightly lower than those obtained by venipuncture [10]. The reason for the greater capillary red cell counts and hematocrit values in the neonates has been attributed to venous stasis and subsequent seepage of fluid out of the blood during sluggish peripheral circulation [15]. Due to this marked discrepancy between capillary and venous hemoglobin and hematocrit values, it has been stressed that venous samples should be utilized in hematologic determinations in the newborn period to avoid the uncertainties introduced by this factor [7]. However, venipuncture in the neonate can often be difficult and traumatic; some clinicians have used heel warming prior to heel puncture as a means of improving capillary venous sample correlation but there has been no attempt to document the efficacy of such procedure. This report shows that heel warming improves the capillary venous hematocrit correlation. However, the degree of improvement of correlation depends on the age of infants and amount of placental transfusion.

It is known that early and late clamping of the umbilical cords at birth greatly influences the hematocrit values during the first few days of life [2, 4, 17]. By serially measuring the hematocrits of simultaneously obtained venous and capillary blood in early and late clamped infants during the first 5 days of life, this investigation shows that late cord clamping at birth produces a hemodynamic situation whereby the infants adjusted by a process of fluid transudations in the capillary beds resulting in the alteration in the capillary as well as venous hematocrits.

Materials and Methods

The subjects of this study were 60 f ll term newborn infants born fter 38 to 42 weeks of gestation following uncomplicated pregnancies, labors and deliveries t the Southern Maternity Hospital (Sodra Barn

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TABLE 1 *Comparison of hematocrits values on simultaneously obtained femoral and scalp venous blood in 13 newborn infants of various age groups*

Infant number	Age	Birth weight (g)	Early or late clamped	Hematocrit in %: Femoral vein	Hematocrit in %: Scalp vein	Diff. %
1	15 min	3120	early	52	50	+2
2	27 min	3960	late	60	58	+2
3	30 min	3200	lat	62	6_	0
4	41 min	4020	late	68	67	+1
5	2 hr	3900	late	[illegible]	70	−1
6	4 hr	3170	late	55	56	−1
7	6 hr	4600	late	68	66	+2
8	7 hr	2600	late	[illegible]	69	0
9	13 hr	3850	late	63	67	−4
10	22 hr	3200	late	51	54	−3
11	22 hr	3750	late	61	61	0
12	48 hr	[illegible]	lat	64	64	0
13	5 d	3240	early	[illegible]	60	−1
			Mean	61.5	61.8	−0.3

bordshuset) Stockholm, Sweden. The infants weighed 2650 to 4600 g at birth; 28 of them were male and 22 were female. Only 3 mothers received intermittent nitrous oxide inhalation about ½ hour before delivery; none of the mothers received analgesia; all infants were normal at birth and none developed any illness during their stay in the nursery.

The infant were divided into two groups depending on when the umbilical cords were clamped at birth:

Group I. Early clamped group (10 infants). The cords were clamped immediately after the delivery of infant buttocks regardless of onset of respiration and cry. The erage time of cord clamping was 7.3 sec (range: 0 t 30 sec). Cords of 16 infants were clamped within 5 sec two at 15 sec, one t 25 sec and one at 30 sec after birth.

Group II. Late clamped group (40 m fant). The cords of these infants were tied and severed after umbilical arterial pulsations had ceased. N attempt was made t milk the cord before clamping. The mean time of cord clamping was 3 min and 35 sec after birth. (Range f 2 min and 30 sec to 5 min). At the time of birth and before the cords were clamped, the infants had on the delivery bed about 10 cm below the introitus of the mothers.

A total of one hundred and eleven sets of blood samples were simultaneously obtained from the scalp veins, unwarmed and warmed heels at ¼ 2 to 6, 12 to 24 hours and 3 to 5 days of life. One to 2 ml of blood were drawn from the scalp vein into a 2 ml syringe containing just enough heparin solution (5000 I.U./ml) to wet its inner wall. Scalp veins were selected as the site of venipuncture because of convenience and safety. In separat study hematocrits of 13 infants of various age groups were measured in the simultaneously obtained femoral and scalp venous blood. Results show that scalp and femoral venous bloods had almost the same hematocrit values (Table 1). A few seconds after enipuncture capillary blood was obtained by heel puncture using blood lancet (Serasharp, propper Mfg Co., N Y) and blood collected into a preheparinized microcapillary tube. In the meantime the opposit heel was immersed in a warm water bath (water temperature 40–42°C) for 3 to 4 min. The skin temperature of both heels were measured by means of thermocouple (Electro laboratoriet, Copenhagen). The skin tem

TABLE 2. *Simultaneous capillary (from warmed and unwarmed heels) and venous hematocrits in 40 infants with late and 20 infants with early cord clamping during the first 5 days of life.*

M = Mean. SEM = Standard error of the mean. N = Number of determination.

Age		Late clamped group: Capillary hematocrit % (warmed heel)	Late clamped group: Capillary hematocrit % (unwarmed heel)	Late clamped group: Venous hematocrit %	Early clamped group: Capillary hematocrit % (warmed heel)	Early clamped group: Capillary hematocrit % (unwarmed heel)	Early clamped group: Venous hematocrit %
½ hour	M	63	67	57 7	59.8	59.7	53.3
	SEM	1.94	1 60	1.59	1.48	1 65	1 42
	N	11	11	11	11	11	11
2–6 hours	M	67	71	63	58.5	59 1	54.5
	SEM	1 16	1 11	1.18	1.90	1 78	1 72
	N	17	17	17	11	11	11
12 24 hours	M	61 4	64.4	57.3	52	54	48.8
	SEM	1 40	1.32	1 41	1.86	1 77	1 61
	N	17	17	17	13	13	13
3–5 days	M	62	63.5	60	53.5	55	51
	SEM	0.85	0.88	1.28	1 76	1 74	1.55
	N	15	15	15	16	16	16

Number of infants 40 Number of infant 20

perature on the unwarmed heel ranged from 26.8 to 34 6°C, depending on age environmental temperatures and amount of placental transfusion. It has been shown that the cutaneous temperature in the extremities of early clamped infants were significantly lower than the late clamped infants during the first hours of life [13]. The skin temperature on the artificially warmed heels ranged from 35°C to 37°C immediately after warming and fell to 33 to 34°C after blood sampling was done The capillary blood was obtained from the warmed heels in the same manner as in unwarmed heels described previously In all instances, free flow of blood was accomplished with minimal heel squeezing If excessive squeezing was required to obtain blood, a new puncture was done

Hematocrits were measured on each sample by the technique described by Guest & Siler [8]. The microcapillary tube containing heparinized blood was centrifuged at 11 000 Rpm for 4 to 5 min and the packed red cells were read on the hematocrit Reading chart (A R Thomas Co., Philadelphia) No attempt was made to correct the trapped plasma in the red cell column. Double determinations for capillary hematocrit measurement were performed on eleven infant to determine the errors incurred during sampling and instrumentation The error was found to be ±3.35% of the observed hematocrit values.

Results

The results of this study were summarized in Table 2 In the late clamped group of infants the capillary hematocrits from the unwarmed heels showed an initial value of 67% at 30 min, rose to 71% at 2 to 6 hours, then fell to 64 4% at 12 to 24 hours and stabilized at 63.5% at 3 to 5 days of age The venous hematocrits measured at the same time showed similar trend, the value being 57 7 at 30

TABLE 3. *Significance of differences of capillary and venous hematocrits in different age periods in early and late clamped infants during the first 5 days of life.*

	Late clamped group		Early lamped group	
	Capillary hematocrit (unwarmed)	Venous hematocrit	Capillary hematocrit (unwarmed)	Venous hematocrit
30 min vs. 2–6 hours	+ increase	+ + increase	– –	– –
2–6 hours vs. 12–24 hours	+ + + + decrease	+ + + decrease	+ decrease	+ decrease
12–24 hours vs. 3–5 days	– –	– –	– –	– –

–$p > .05$. +$p < .05$. + +$p = .01$ .02.
+ + +$p = .001$ .01 + + + +$p = < .001$.

min, 63% at 2 to 6 hours, 57.8% at 12 to 24 hours and remained at 60% at 3 to 5 days of life. Warming the heel before sampling brought down the capillary hematocrit values of the unwarmed heels from 67% to 63% at 30 min, 71 to 67% t 2 to 6 hours, 64 4 to 61 4% at 12 to 24 hours and 65.5 to 62% at 3 to 5 days old.

In the early clamped group the capillary hematocrit of the unwarmed heels was 59 7% at ½ hour 59% at 2 to 6 hours, 54% at 12 to 24 hours and 55% at 3 to 5 days of age. Except for the slight decline in its value between 2 to 6 hours and 12 to 24 hours of life ($p < .05$) there were no significant alterations in the unwarmed capillary hematocrit in the early clamped infants when plotted against ge The venous hematocrits were 53.3%, 54.5%, 48.8% and 51% at ½ hour 2 to 6 hours, 12 to 24 hours and 3 to 5 days of age respectively Again, except for the slight decline between 2 to 6 hours and 12 to 4 hours of age ($p < .05$), there were no significant alterations in the venous hematocrits in the early clamped infants when correlated with age (Table 3) In contrast to the late clamped group of in fants, where heel warming before puncture apparently improved the capillary venous hematocrit correlation the procedure produced only slight change in the early clamped infants. Although it should be pointed out that the capillary venous hematocrit difference in the early clamped group were not as pronounced as in the late clamped infants (Table 2)

In Fig 1 the individual values of the unwarmed capillary hematocrits were plotted against age to show the rise be tween ½ hour and * to 6 hours of life and the subsequent fall t the age of 1 to 24 hours of life in the late clamped group of infants and the relatively unchanged ca pillary hematocrit value in the early clamped infants. The mean values and stand ard error of the means of the simultane ously measured venous hematocrits in late and early clamped infants were also in cluded in the graph to depict the striking similarity of changes with age when com

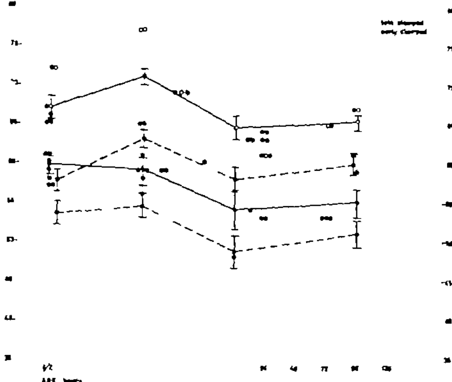


Fig. 1 Scattergram of unwarmed capillary hematocrits serially measured in 40 infants whose umbilical cords were clamped late and 20 infants whose cords were clamped immediately after birth; solid lines connect their mean values. Dotted lines represent the means and standard error of the means of simultaneously obtained venous hematocrits.

pared with their corresponding capillary values.

In Fig 2-5 the correlation between the unwarmed capillary warmed capillary and venous hematocrits at ½ 2 to 6 hours, 12 to 24 hours and 3 to 5 days of age were shown. In the graph, the solid line represents the theoretical equality line while the dotted lines were the standard errors from sampling and instrumentation (±3.25%) At age ½ hour poor correlation exists between the unwarmed capillary and venous hematocrits in the late clamped infants; heel warming slightly improved the correlation. In early clamped group the disparity between capillary (unwarmed heel) and venous hematocrits was less pronounced and heel warming did not alter the difference (Fig 2) At 2 to 6 hours, the discrepancy between the unwarmed capillary and venous hematocrits in the late clamped infants was still pronounced with only 3 out of 17 paired samples fell within the range of the venous values. On heel warming 8 out of 17 capillary hematocrits compared well with the venous values. In early clamped infants 7 out of 11 unwarmed capillary hematocrits correlated well with venous values warming did not increase the number of correlation (Fig. 3)

At 1 to 24 hours of age, only 3 out of

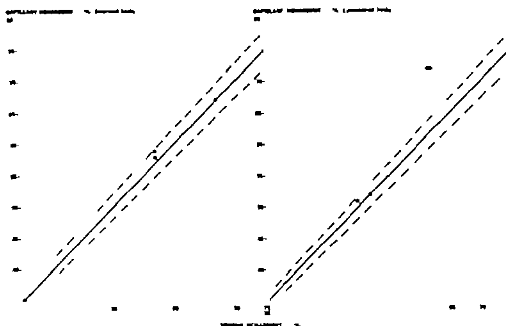


Fig. 2. Warmed and unwarmed capillary hematocrits versus venous hematocrits in 22 infants half hour of age. Solid lines are theoretical equality lines (y =). Dotted lines represent errors incurred during sampling and instrumentation. Open circles are late clamped and closed circles are early clamped infants.

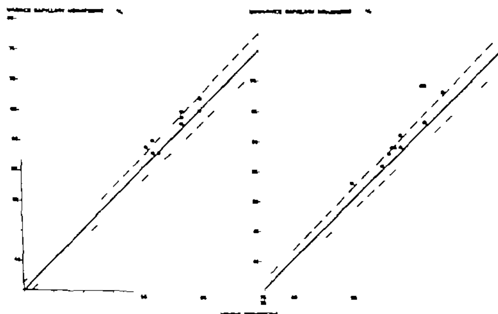


Fig. 3. Warmed and unwarmed capillary hematocrits versus venous hematocrits in [illegible] infants 2 to 6 hours of age. Legend for solid lines, dotted lines and circles are presented in Fig. 2.

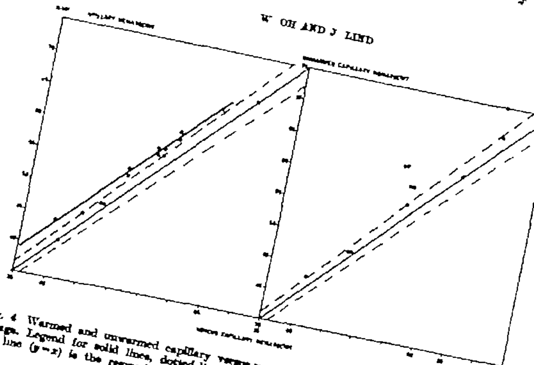


Fig. 4. Warmed and unwarmed capillary versus venous hematocrits in 30 infants 12 to 24 hours of age. Legend for solid lines, dotted lines and circles are presented in Fig. 2. Solid line to the left line $(y=x)$ is the regression line which can be derived from the formula $y=2.851+0.994\,x$. This line is significantly different from the line $y=x$.

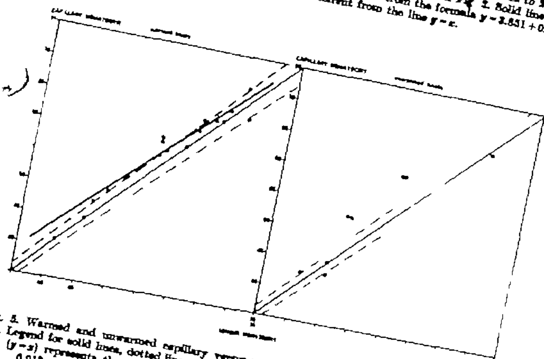


Fig. 5. Warmed and unwarmed capillary versus venous hematocrits in 31 infants 3 to 5 days of age. Legend for solid lines, dotted lines and circles are presented in Fig. 2. Solid line to the left of line $(y=x)$ represents the regression line which can be derived from the formula $y=7.101+0.912\,x$. This line is significantly different from the theoretical equality line $(y=x)$.

17 capillary hematocrits correlates well with the venous values in the late clamped group heel warming increased the number to 8 out of 17 In early clamped group 4 out of 13 capillary hematocrits correlate with the venous values, warming increased the number to 7 out of 13 For both groups of infants the regression line ($y = 3.851 + .994\ x$) calculated for the paired samples of warmed capillary and venous hematocrits is significantly different from the theoretical equality line ($y = x$) Given a warmed capillary hematocrit values (y) the 95 % confidence limits for the corresponding venous values (x) could be derived from the formula

$$3\ 07 + 835y \pm 5.335 \sqrt{\frac{1}{30} + \frac{(y - 57\ 433)^2}{1777.307}}$$

At 3 to 5 days old, only 5 of 15 late clamped infants have unwarmed capillary hematocrits that correlate well with venous values; with heel warming the correlated paired samples were 10 out of 15

In early clamped infants 5 out of 16 unwarmed capillary hematocrits correlates with venous hematocrit and warming reduce the number of uncorrelated paired samples to 5 The regression line ($y = 7\ 101 + .913\ x$) for the paired samples of warmed capillary and venous hematocrits of both early and late clamped infants was still significantly different from the theoretical equality line ($y = x$) with a known warmed capillary hematocrits (y), th 95 % confidence limits for the corresponding venous hematocrits (x) could be calculated by using the formula

$$1\ 315 + .938y \pm 5.738 \sqrt{\frac{1}{31} + \frac{(y - 57\ 710)^2}{1546\ 387}}$$

Discussion

Since heel puncture was the source of capillary blood in this study venous blood obtained from one of the vessels in the lower extremities would be the ideal reference point in comparing capillary venous hematocrit difference. However as femoral venipuncture in the newborn infant is not an entirely innocuous procedure blood from one of the scalp veins was utilized. A preliminary study has shown that venous blood simultaneously obtained from the femoral and scalp vein showed almost similar hematocrit values in various age groups (Table 1) This result indicates that blood obtained from different venous sampling sites would probably reveal similar hematocrit values which represent the systemic venous hematocrit unrelated to the capillary areas the particular venous vessels drained.

The initial rise in unwarmed capillary hematocrits between ½ hour and * to 6 hours of age in infants given placental transfusion (Table * and Fig 1) probably represents fluid transudation in the capillary bed of the lower extremities and other parts of the body in response to vascular distension resulting from the 61 % increase in blood volume as shown by Usher Shephard & Lind [17]. The simultaneous decline in blood and plasma volume as observed by these same workers and by Steele [16] also conforms with this rise in capillary hematocrits during this period of neonatal life The subsequent fall of capillary hematocrits between _ to 6 hours and 1 to 4 hours of age indicates a process of fluid reabsorption in th capillary microcirculatory system into the intravascular compartment as a means of re-

storing the circulatory blood volume in response to the increasing circulatory demand in the visceral organs such as gastrointestinal tracts which is assuming a more active metabolic role secondary to beginning allimentation. It is logical to assume that the similar alteration in venous hematocrit values during the first 24 hours of life as observed in our study and those of others [2, 4, 17] is a reflection of changes occurring in the capillary system.

In infants deprived of placental transfusion through immediate cord clamping, no alterations in the capillary and venous hematocrit were observed when the values were plotted against age since these infants were not subjected to the same hemodynamic situations that the late clamped group of infants encountered, such observation was not unexpected.

The reason for the capillary hematocrit being higher than the simultaneously obtained venous value in the newborn is not well known. It has been demonstrated in the experimental animals that prolonged ... etic nervous system stimulation produces vasoconstriction of the precapillary sphincter [5 11], in the newborn infants Celander & Mårild [1] have shown that in response to lower ambient temperature and *concomitant increase in sympathetic* activity the capillary filtration coefficient which is a measurement of available capillary surface areas, increases This increase in the capillary surface area was considered as a defense mechanism in the newborn infants in response to reduced blood flow due to vasoconstriction, so that more capillary exchange might occur to insure proper tissue metabolism The reduced regional blood flow secondary to sympathetic activity combined with poor peripheral circulation through increased capillary surface areas favor net movement of fluid out of the intravascular space in the capillary areas, accounting for the capillary venous hematocrit difference in the newborn. However if this were the sole operating factor the capillary venous hematocrit difference would be more profound in the early clamped infants since they have a lower blood volume systemic blood pressure and cutaneous temperature [13 14] probably requiring greater sympathetic nervous system activity But on the contrary our results showed a lower capillary venous hematocrit difference in early clamped than the late clamped infants. It seems likely that the vasoconstriction and reduced local blood flow do play an important role in the production of capillary venous hematocrit difference in the newborn infants; however in the early clamped infants, who were given small amount of placental blood transfusion, the findings in capillary and venous hemotocrits suggest that they paradoxically maintained their available blood and plasma volume within the intravascular space with minimal fluid transudation.

It is of practical and clinical importance to document the effects of local heel warming on the capillary venous hematocrit correlation in the newborn infants. It is obvious from Fig –5 that there is a marked discrepancy between the capillary and venous hematocrits when the former was obtained from the unwarmed heels. Heel warming prior to heel puncture improved the capillary venous hematocrit correlation, and the procedure becomes more effective in the older infants. How

ever it should be emphasized that absolute correlation between venous and capillary hematocrits could not be achieved by heel warming even in infants 3 to 5 days of age This differs from the findings of Gandy *et al.* who recently showed that capillary pH and PCO obtained from warmed heel compared well with simultaneously determined arterial values in infants over one hour of age [6]. Nevertheless, clinicians who have to follow serial hematologic measurements such as hemoglobin, hematocrits or red blood cell counts in the neonates could still utilize warmed capillary blood samples when venipuncture for venous samples is impractical, but should keep in mind that the correlation between the warmed capillary and venous hematocrits is not absolute Moreover the more exact venous hematocrit corresponding the observed capillary value could be calculated from the regression line shown in Fig 4 and 5 Given a warmed capillary hematocrit values (y) the 95% confidence limits of the corresponding venous values could also be calculated from the appropriate formula listed elsewhere in this paper

The improvement of capillary venous hematocrit correlation upon local warming is probably a result of enhanced local circulation. Landis has proven that warming the skin of human adults increase both arterial and venous capillary pressure with arterial limb pressure being higher than the venous [9]; this physiologic phenomenon facilitates local circulation by increase in the speed of flow and venous return with reabsorption of plasma in the intracellular space into the vascular space. This mechanism apparently operates in infants allowed placental blood transfusion and with high capillary venous hematocrit difference. But in the early clamped infants the capillary venous hematocrit discrepancy was not pronounced, so that improving the local circulation by warming would equally accomplish less.

Summary and Conclusion

Hematocrit values were measured on one hundred and eleven sets of capillary (from unwarmed and warmed heels) and venous blood samples obtained simultaneously during the first 5 days of life from 60 full term newborn infants, 40 of which the umbilical cords were clamped late and *0 clamped early at the time of birth.

In the late clamped infants the capillary hematocrits showed an initial rise during the first 6 hours of life seemingly due to fluid transudation in the capillary beds, followed by a fall at 12 to *4 hours of age due to a subsequent fluid reabsorption into the vascular space in response to increasing circulatory demands in the visceral organs. In the early clamped infants, the capillary hematocrits remained stable during the first 6 hours but a slight decline was observed at 1_ to 24 hours of age The simultaneously measured venous hematocrits of both late and early clamped infants plotted against age revealed a strikingly similar pattern of alterations.

A marked capillary venous hematocrit difference was observed in the lat clamped infants and to a much lesser extent in the early clamped infants during the first 5 days of life with the venous being lower than the capillary values. Warming the heels prior to capillary sampling improves the capillary venous hematocrit correlations in the late clamped infants and the improvement achieved by this procedure

increases as the infant becomes older In the early clamped infants heel warming produces relatively less effects because there was less capillary venous hematocrit discrepancy initially

In infants over 12 hours of age where venipuncture is difficult or inadvisable capillary blood samples obtained from warmed heels could be used for hematocrit measurements. However the hematocrit reading of the warmed capillary samples do not exactly correspond with the venous values and the approximate venous reading could be estimated by using the regression lines derived from our samples, and their 95 % confidence limit could be calculated from the appropriate formulas.

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References

1 Celander, O and Mellin K.: Regional circulation and capillary filtration in relation t capillary exchange in the foot and calf of the newborn infant. *Acta Pediat Scand, 51* 385, 1962.

2. Conr R. L. and Priestlova, H.: Placental transfusion and fluid metabolism on the first day of life. *Arch Dis Child, 39* 363 1964.

3. De Marsh, Q B., Alt B. L. and Windle, W F Factors influencing the blood picture of the newborn. *Amer J Dis Child, 75* 860, 1948.

4 De Marsh, Q B., Alt, H. L. and Windle, W F *Effect of depriving infant of its placental blood on blood picture during first week of life. JAMA 116* 2568, 1941.

5. Folkow B. and Mellander, S. Aspects of the nervous control of the precapillary sphincters with regard to the capillary exchange. *Acta Physiol Scand, Suppl 175* 52, 1960

6. Gandy G., Grann L., Cunningham, N Adamsons, K. Jr. and James, L. S. The validity of pH and PCO measurements in capillary samples in sick and healthy newborn infants. *Pediatrics 34* 192, 1964.

7 Gairdner, D *The Hematology of I fancy* In *Recent Advances in Pediatrics*, 2nd Ed. Gardner D Ed. 1958.

8. Guest G M. and Siler, V E. Centrifuge method for determination of volume of cells in blood. *J Lab Clin Med 19* 757 1934.

9 Landis, E. M.: Microinjection studies of capillary blood pressure in human skin. *Heart, 15* 209, 1930.

10. McGovern J J J ... , A. R. and Steinberg A. G The hematocrit of capillary blood. *New Engl J Med 253* 308, 1955.

11 Mellander, S.: Comparative studies of the adrenergic neurohormonal control of resistance and capacitance blood vessels in the cat. *Acta Physiol Scand, Suppl 176* 1960

12. Oettinger, L. Jr. and Mills, W B Simultaneous capillary and venous hemoglobin determinations in the newborn infant. *J Pediat, 35* 362, 1949

13. Oh, W *and Lind J Regulation of body* temperature in newborn inf nts with early and late cord clamping In manuscript.

14. Oh, W Lind J and Gessner, I: Circulatory and respiratory adaptation to early and late cord clamping in newborn infants. *Acta Pediat Scand, 55* 17 1966.

15. Smith, C. A.: *The Physiology of the Newborn Infant.* 3rd Ed. Blackwell Scientific Publications, Oxford, 1959 p. 157

16. Steele, M. W Plasma volume changes in the neonate, *Amer J Dis Child, 103* 10 1962

17 Usher, R., Shephard M. and Lind J The blood volume of the newborn infant and placental transfusion. *Acta Pediat Scand, 52* 497 1963.

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Adolescent Colloid Goitre

by LARS R. NILSSON

The term adolescent goitre is used to designate a diffuse swelling of the thyroid at puberty and generally believed to be a special type of goitre. It is ascribed to compensatory hyperplasia to meet the increased demand for thyroid hormone during this period of life [1, 22], but it is questionable whether this explanation is sufficient. The possibility of some co-existing disorder of the thyroid has also been suggested [22]. However goitre at puberty is not a uniform condition. Auto-immune thyroiditis has recently proved a common cause of goitre at this age [1, 10, 14, 16, 19]. When the goitre is endemic, it usually appears during adolescence [ ]. A thyroid enlargement at puberty as well as goitre making its first appearance later in life may also be due to extrinsic factors other than iodine deficiency. Congenital disorders of the synthesis of the thyroid hormone may sometimes be so mild as not to cause demonstrable goitre until puberty [15]. Though many causes of goitre are known, there is still a large group in which the pathogenesis is obscure. To this group belong those cases of goitre which occur at puberty and afterwards disappear spontaneously.

This paper is concerned with a clinical study of a series of adolescent goitre of unknown pathogenesis. On the basis of the palpatory and the microscopic findings this type is referred to here as adolescent colloid goitre to distinguish it from other forms of goitre during puberty.

Material

Between September 1959 and October 1963 all together 82 cases of goitre were seen in patients, aged 9 to 17 years, at the Children's Hospital of Gothenburg. On the basis of clinical evaluation, radioiodine studies, immunological methods and cytological aspiration biopsies the material was divided into various diagnostic groups (Table 1). Autoimmune thyroiditis has been dealt with in previous reports [4, 16]. The present study was concerned with the patients with goitre of obscure origin. Thirty-nine (37 girls and 2 boys) of the patients had simple colloid goitre. This number included three (two were sisters) in whom the gland was cystic.

TABLE 1. *Different kinds of goitre in patients aged 9–17 years seen at the Gothenburg Children's Hospital between Sept 1959 and October 1963*

	Girls	Boys	Total
Thyrotoxicosis	3	1	4
Auto-immune thyroiditis	30	3	33
Inborn error of thyroid hormonogenesis	2	3	5
Iodide goitre	1	—	1
Cystic colloid goitre	3	—	3
Simple colloid goitre	34	2	36
	73	9	82

Table 2 *Data about 39 cas*

Case No.	Init.	Sex	Age: Exam.	Age: Menarche	Known duration of goitre	Size of goitre	Clin. thyroid status	(+15 to −15%) BMR	(4–8 μg%) PBI	I^{131} uptake (20–50%): 7	24	48 h
1	U W	F	10		4 yrs	M	eu	+4	5.2	40	48	48
2	L S.	F	10½	12½	1 mth	M	eu	−2, +4	5.7	32	41	45
3	Y P	F	10½	10½	6 mths	M	eu	+10 +8	6.2	4	48	60
4	M. A.	F	10½	10½	1 mth	L	eu	+3, −7	6.6	31	40	42
5	E. A.	F	11	12	1 mth	S	eu		7 7	42	46	52
6	A. M. O.	F	11½	13	3 mths	S	eu	−11	9.0	8	18	22
7	U B.	F	13½	13½	1 mth	M	eu	+2, −13	3.2	67	74	73
			14			M	eu	−7	7 4	38	46	48
8	L. L.	F	11½		1 mth	S	eu	+16	6.3	23	36	38
9	B. S.	F	12	13	1 mth	M	eu	+8	7.2	40	44	51
10	B B.	F	13½	13	1 mth	S	eu	+7	7.5	42	46	51
11	G L. D	F	13½	13½	1 mth	S	eu	−12, −14	6.2	49	61	62
12	U O	F	14	13½	1 yr	M	eu	−22, −23	4.3	23	30	41
13	J G	F	14	13½	2 mths	M	eu	+4, +6	4.5	41	51	47
14	L. A.	F	14	14	1 yr	M	eu	−12, −4	6.0	58	64	61
15	E. B.	F	14	10	1 mth	S	eu	−16	4.8	45	53	52
16	B M.	F	14	12	?	M	eu		5.4	22	36	41
17	L N	F	14½		1 mth	S	eu	+4	7.0	41	52	51
18	S. S.	F	14½	13½	1½ yrs	L	eu	+6	8.5	29	36	36
19	G. A.	F	15	12½	4 mths	S	eu	−5	5.3	24	34	35
20	L. M.	F	15	11½	3 yrs	M	eu	±0	5.2	38	46	46
21	B S.	F	15	14½	1 mth	M	eu	±0 −1	2.9	23	34	33
			17½						4.5			
22	U B	F	15	15	1 mth	L	eu	+3	6.4	55	59	54
23	P S.	F	15½	11	?	S	eu	−2	6.1	25	35	35
24	A. S.	F	15½	12	2½ yrs	M	eu	+2, −2	5.2	36	39	44
25	B. L.	F	15½	13	1 mth	M	eu	+8	6.5	24	36	37
26	L. B.	F	15½	12	2 mths	M	eu	−7	7.2	35	49	49

Follow up		Remarks
Y	Course of goitre	
3	None	Rheumatic fever at 15 yrs. Mat.grandmother thyrotox.
3	Regression (?complete)	Mother ?thyrotox. Four mat. rels goitre (1 thyrotox.)
½	Unchanged	
3½	Decreased	Pat.aunt and her daughter op. thyrotox. Another aunt goitre.
1½	Unchanged	Iron deficiency anaemia. One pat., 1 mat. rel? thyrotox.
1½	Unchanged	
1	Slightly decreased	Mother slight puberty goitre, mat.grandmother and her mother goitre
½	Unchanged	Two mat rels goitre
1	Fluctuating size	
1	Slightly decreased	Bronchial asthma. Mother thyrotox. + asthma.
1½	Initial regression—later relapse of cyst on treatment with thyroxine	Mother small goitre, mat. aunt goitre, 2 pat. rels goitre.
3½	Y goitre on treatment with thyroxine	Resections of cystic thyroid tissue performed at 14 yrs of age in both sisters
3	Still persisting on treatment with thyroxine	Six pat. rels, 1 mat.rel. goitre

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renal excretion of radioiodine were determined by the conventional technique. Amberlit Ira resin 400 was used for the separation of protein-bound and free I^{131} in plasma.

Thyrotropin stimulation tests Actyron® (Ferring, Malmö, Sweden) was injected intramuscularly in a dose of 0.15 units/kg bodyweight. In 3 cases the iodine uptake by the thyroid was determined before and 24 hours after TSH-injection. The TSH-effect on the PBI^{131}-levels in plasma during radioiodine tests was studied in 10 cases. The PBI^{131} level was determined both at 48 and at 72 hours, and the TSH-injection was given 18 to 24 hours before collection of the second blood sample. The ordinary nonradioactive PBI level was examined 1 f and 18–24 hours after H-injection in cases.

Table 2 (Continued)

Case No.	Init.	Sex	Age at Exam.	Age at Men-arche	Known dura tion of goitre	Size of goitre	Clin. thyroid status	(+15 to −15%) BMR	(4–8 μg%) PBI	I[131] uptake 7	24	48 hr (20–50%)
27	B. S.	F	15½	13	3½ yrs	M	eu	−2, −2	4.0	22	33	32
28	A Ö	F	16	14	?	S	eu	−22, −30	7.4	30	47	48
29	B. S.	F	16	13	3 mths	S	eu		5.9	23	37	35
30	B. A.	F	16	13	4 yrs	L	eu	−12, −14	7.8	61	65	65
31	B. S.	F	16	1 ½	1 mth	S	eu		9.8	53	64	66
32	L. S.	F	16½	13	2½ yrs	M	eu		6.9	33	50	49
33	B. K.	F	16½	12½	1½ yrs	S	eu	+3	5.4	43	49	52
34	G A.	F	17½	13	1 mth	S	eu	−12, −17	5.2	30	39	39
35	B. S.	M	10		1 yr	S	eu	+7	5.3	15	26	27
36	P S.	M	15		4 mths	S	eu	+5, +16	5.1	13	16	14
37	E. L.	F	13	12½	1 yr	M cystic	eu	−7	7 7	24	37	37
38	M. N	F (sibs)	15	13½	1½ yrs	L cystic	eu	±0	6.6	19	28	27
39	M. L. N	F (sibs)	18	14	4 yrs	M cystic	eu					

Examined with tanned red cell haemagglutination and complement-fixation only

Two of the patients had taken a cough mixture containing iodide (Case 6) or ammonium chloride (Case 36) but the medicine had been taken in such amounts and at such a time that it could not be regarded as the only cause of the goitre All patient except one were from Gothenburg a seaport with about 420 000 inhabitants situated on the west coast of Sweden, where endemic iodine deficiency is unknown. There is only *one* children's hospital in the town.

Methods

Cytological aspiration biopsies were performed by Dr Sigvard Persson, Medical Department I, the Sahlgren's Hospital Gothenburg The technique and results have been reported elsewhere [17]. Biopsy specimens were obtained from 28 of the patients and in 3 (Cases 14, 38 and 39) resection of the goitre had been done and examined histologically In 6 patients (Cases 5, 6, 21 24 27 and 28 in Table 2) the goitre had disappeared spontaneously before the introduction of the fine-needle aspiration technique and 3 patients (Cases 19 23 and 34) would not consent to biopsy

Serological methods The immunological examination was performed mainly by Dr Deborah Doniach, Middlesex Hospital Medical School London Four sera were examined by Professor Astrid Fagreus Department of Virology State Bacteriological Laboratory Stockholm, and 7 sera by Dr Stig Bertil Nilsson, Blood Donor Service Lund University Hospital. In 3 patients no ant

(…inary …retion) 24–48 hrs (…–60%)	(0–0.20 %/l) PBI[131] 48 hrs	Thyroid anti-bodies	Follow up Y	Course of goitre	Remarks
7	0.05	—	3	None	Rheumatic fever at 15 yrs. Mat.grandmother thyrotox.
1		—	3	Regression (?complete)	Mother ?thyrotox. Four mat. rels goitre (1 thyrotox.)
3	0.02	—	½	Unchanged	
1	0.20	—	3½	Decreased	Pat.aunt and her daughter op. thyrotox. Another aunt goitre.
1	Not measurable	—	1½	Unchanged	Iron deficiency anaemia. One pat., 1 mat. rel? thyrot x.
1	0.13	+	1½	Unchanged	
3	0.03	—	1	Slightly decreased	Mother slight puberty goitre mat.grandmother and her mother goitre
1	0.02	—	2½	Unchanged	Two mat.rels goitre.
5	0.06	—	1	Fluctuating size	
4	0.03	—	1	Slightly decreased	Bronchial asthma. Mother thyrotox. + asthma.
1	Not measurable	+	1½	Initial regression—later relapse of cyst on treatment with thyroxine	Mother small goitre mat. aunt goitre, 2 pat. rels goitre.
3	0.03	—	3½	↑ goitre on treatment with thyroxine	Resections of cystic thyroid tissue performed at 14 yrs of age in both sisters.
		—	3	Still persisting on treatment with thyroxine	Six pat. rels, 1 mat.rel. goitre.

body tests were performed. References to the methods were given in a recent paper on immunological findings in auto-immune thyroiditis [4]. The methods used were: tanned red cell haemagglutination test (TRC-test) for detecting antibodies to thyroglobulin, immunofluorescence (Coons technique) for detecting antibodies against the cytoplasmic antigen and the second colloid antigen (CA 2) and complement fixation test (CF-test) for titration of antibodies to the cytoplasmic antigen.

Radioiodine tests. Radioiodine was administered orally with the patient in the postabsorptive state. The dose used was 0.10–0.20 microC in order to be able to determine the protein bound radioactivity in plasma at 48 hours (PBI[131]-48 hrs). Thyroid uptake and renal excretion of radioiodine were determined by the conventional technique. Amberlite Ira resin 400 was used for the separation of protein bound and free I[131] in plasma.

Thyrotropic stimulation tests. Actyron® ("Ferring", Malmö, Sweden) was injected intramuscularly in a dose of 0.15 units/kg bodyweight. In 2 cases the iodine uptake by the thyroid was determined before and 24 hours after TSH-injection. The TSH-effect on the PBI[131]-levels in plasma during radioiodine tests was studied in 10 cases. The PBI[131]-level was determined both at 48 and at 72 hours, and the TSH injection was given 18 to 24 hours before collection of the second blood sample. The ordinary nonradioactive PBI level was examined before and 18–24 hours after TSH-injection in 6 cases.

The thyroxine degradation rate was examined in one patient according to the technique described by Sterling, Lashof & Man [21].

The protein-bound iodine (PBI) was determined with a modified Barker method [20].

Results

Onset and Clinical Manifestations

Goitre had been discovered by the patients themselves or by their parents in 22 cases at routine school medical examination in 9 and accidentally at examination for some other disease in 8. The age at which the goitre was first observed varied from 6 to 17 years, mostly between 11 and 15 years, with a mean of 13–13½ years (Fig. 1) The onset occurred at or near menarche in more than half of the cases (Fig 2) It is possible that also some of the cases discovered at varying periods after menarche had in reality occurred at puberty but had escaped detection. The median age at menarche was 12 years and 10 months (range 10 years and 2 months to 14 years and 11 months) and the mean age, 12 years and 8 months

Most of the goitres were judged as small to medium-sized (Table 2) They had a smooth surface and were homogeneous, elastic and of fairly firm consistence. Some of the goitres felt soft others distended, as if they were full of colloid. In a few

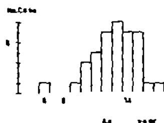


Fig 1 Distribution of the cases according to age at onset.

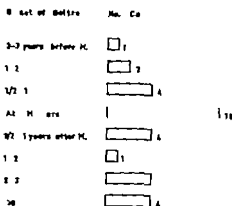


Fig. 2. The relation between menarche and onset of goitre.

large goitres the consistence of some parts differed from the rest of the gland suggesting the possibility of lymphoid thyroiditis Three goitres were cystic. Two sisters had previously been operated upon for cystic lesions in the neck, which had later recurred.

Goitre was the only objective sign of thyroid disease. There were no clinical signs of hypothyroidism. The patients were of normal height and weight. Two patients with low BMR complained of fatigue and intolerance to cold, but otherwise showed no evidence of hypofunction. Twenty seven of the 39 patients were symptom free and the remaining 12 had mainly mild diffuse symptoms not clearly related to thyroid dysfunction (Table 3) One patient (Case 22) with symptoms suggesting toxic goitre proved to have a grave mental disorder that was probably not related to goitre.

Cytological picture

The cytological picture of adolescent colloid goitre has been described and illustrated in a previous paper [17] In most

TABLE 3. *Various symptoms and associated disorders in 39 cases of adolescent colloid goitre.*

	No. of cases
Neck discomfort	4
Fatigue	5
Cold sensitivity	3
Increased sweating	2
Palpitations	2
Nervousness	4
Headache	2
Slight eye-signs	1
N symptoms ascribable to thyroid disease	27
Associated disorders	
Alopecia areata	1
Asthma bronchiale	1
Recidivating bronchitis	1
Recidivating tonsillitis	1
Urinary tract infection	1
Rheumatic fever	1
Iron deficiency anaemia	1
Hereditary hearing defect	1
Pyrohocurosis	1

of the cases smears showed abundant colloid, but in a few cases it was only scanty The follicular epithelium was largely of uniform appearance with fairly large nuclei containing nucleoles, vacuolated cytoplasm and numerous paravacuolar granules which were interpreted as signs of secretory activity No definitely degenerative changes were observed and there was no accumulation of lymphoid elements.

Two cystic goitres were punctured. Their contents were watery Smears from one of the cysts in one of the sisters showed phagocytizing cells, cyst phagocytes, and follicular epithelium built up of small dark nuclei containing insignificant amounts of granules. Previous histological examination had shown the picture of a cystic colloid goitre with low epithelium. In the other cystic goitre the firm parts contained an amorphous mass with scattered phagocytizing cells.

Serology

Thyroid antibodies were found in 4 of the 36 sera examined. Thyroglobulin antibodies in a titre of 1/25 were demonstrated in Case 32, and the fluorescent test for antibodies to the second colloidal antigen was weakly positive in 3 cases (Cases 15 18 and 37). Neither in these 4 cases nor in the 3 who were not studied serologically had microscopical examination revealed changes suggesting thyroiditis.

Thyroid function studies

The results in the individual cases are given in Table *

Basal metabolic rate. The values were practically always normal. BMR-values below −10 % were noted in 6 cases, including two in which they were less than −20 %.

Protein-bound iodine (PBI). The values noted were mostly within normal levels and their distribution was even. In 4 patients, including one who had occasionally consumed iodine-containing cough medicine because of recurrent bronchitis the PBI value was slightly increased. In patients it was below normal, but at later control it was found to be normal. No special treatment had been given.

The butanol-extractable iodine (BEI) was determined in 14 patients. The difference between PBI and BEI values exceeded * microg/100 ml plasma in all 14 patients studied except one and the difference was sometimes considerable The method used nd the results of BEI-analyses in this and other types of goitre will be described in a later paper



Fig 3. The thyroidal uptake of radioiodine at 48 hours.

Radioiodine studies The capacity of the goitres to accumulate radioiodine was in general great. The uptake was largely the same at 24 as at 48 hours. The distribution of the 48-hour values is given in Fig 3 Values exceeding 50 % in this geographical area may be regarded as elevated (for adult euthyroid women mean value 37 %, S.D ±13.5 [18]) In one patient (Case 7) with a radioiodine uptake of more than 70 % and subnormal PBI there was reason to suspect that the goitre was due to iodine deficiency By the time of later control the values had become normal without special treatment. Subnormal values were noted in 2 patients who had, however been taking cough medicine containing iodide or ammonium chloride.

The plasma PBI^{131} content at 48 hours was low throughout and was less than 0 10 % of the dose given per litre of plasma in 28 of 35 patients studied (for adult euthyroid women mean value 0.1° %, S.D ±0 09 [18]) With the dose of isotope used however there was no lower normal limit. Only in one case (Case 14) did the PBI^{131} exceed 0.20 %, but a value of 0.24 % cannot be regarded as definitely increased.

Thyrotropin stimulation tests The results are given in Table 4 The uptake of iodine by the thyroid in the 2 cases studied increased markedly after TSH-stimulation. The effect on the level of PBI^{131} was studied in 10 cases. In 7 the PBI^{131} was increased by hundred per cent or more after injection of TSH. The reliability of the values obtained at this level and with the dose of isotope used is, however limited. The non radioactive PBI content increased in all 6 patients studied, but only in 2 of them by more than 2 micrograms per 100 ml plasma.

The thyroxine degradation rate was studied in one case (Case 14). The half time was normal, 7 4 days. Soon after the examination the patient was found to have a plum-sized adenoma in the right thyroid lobe which was extirpated. It could not be excluded that the development of the adenoma had been precipitated by the examination, therefore no further examinations of this type were performed.

Other laboratory studies

Routine determination of the haemoglobin erythrocyte sedimentation rate serum proteins by electrophoresis, and cholesterol gave normal values except for haemoglobin in one patient with iron deficiency anaemia.

TABLE 4 *Effect by exogenous thyrotropin on iodine uptake, PBI[131] and PBI values*

I. *Changes in iodine pool*

		I^{131} uptake in %			PBI^{131} – 48 hr (% dose/litre)
		7 hr	24 hr	48 hr	
Case 15 E. B	before TSH	45	53	52	
	after TSH	70	66	66	
Case 38, M. N	before TSH	19	29	27	0.03
	after TSH	35	41	49	0.04

II. *Changes in PBI[131] -values*

Case No.	Init.	PBI^{131} – 48 hr	PBI^{131} – 72 hr (% dose/litre)
1	U W	0.03	0.06
2.	L. L.	0.07	0.18
9	B. S.	0.01	0.06
17	L. N	0.16	0.28
19	G. A.	0.08	0.21
23.	P S.	0.01	0.06
26.	L. B.	0.10	0.19
29	B. S.	0.03	0.04
33	B. K.	0.08	0.06
36.	P S.	0.05	0.05

III. *Changes in PBI-values*

Case No.	Init.	PBI values in µg % before	after TSH
2.	L. L.	6.2	10.3
9	B. S.	7.3	7.8
17	L. N	7 0	9.5
23.	P S	6.1	7.3
33.	B. K.	5 4	6.1
36.	P S.	5.1	5.7

Follow-up

Observations hitherto made on the spontaneous course of goitre are given in Table 2 and summarized in Table 5. The follow up varied from 1 to 5 years and was usually not long enough to warrant prediction of the definitive prognosis.

The goitre had decreased in size or disappeared in half of the patients. In some of them, however the goitre was still considerable, although they had largely passed puberty In 4 cases it had increased. In one of these 4 patients the goitre had

TABLE 5 *Spontaneous changes in goitre status during the follow up.*

Course of goitre	No. of patients and observation time < 2 yr	> 2 < 3 yr	> 3 yr	Total number
Full regression	1	2	5	8
Decrease	5	1	4	10
Fluctuating size	1	—		3
No change	8	2	1	11
Increase	2		—	4
	17	7	12	36

been detected before puberty one had been delivered of a healthy infant, and one had developed adenoma after determination of the thyroxine degradation rate

In most of the patients reexamined after 3 years the goitre had shown a tendency to regress. Most of the patients in whom no changes were observed had been followed up for less than 2 years (Table 5)

All of the patients have remained euthyroid.

Medication with iodine was tried in three cases without any demonstrable effect. Thyroxine in a dose of 0.1–0.2 mg a day was given to 4 patients including 3 with cystic goitre In 2 of the patients the goitre disappeared. In a third patient the cystic goitre first disappeared, but after reduction of the dose of thyroxine elsewhere the goitre reappeared. In the elder of the two sisters the goitre first decreased but then persisted unchanged despite an increase of the dose of thyroxine to 0.25 mg daily

Family histories

A thorough inquiry was made into the family histories, particularly for the occurrence of thyroid disease and other chronic conditions. The information obtained was published in a previous paper on juvenile auto-immune thyroiditis [4] where a comparison was made between the family histories in three groups of juvenile patients auto-immune thyroiditis adolescent colloid goitre and a control group consisting of patients without goitre cared for at the hospital because of surgical conditions or acute infections. The incidence of goitre among relatives of the two goitre groups was much higher than among those of the control group, but otherwise no significant differences were found. Thyroid disease was noted in 52 relatives of 27 probands of the group auto-immune thyroiditis, in 5. relatives of all together 24 probands in the group adolescent colloid goitre and in 9 relatives of 9 probands in the control group The number of probands in each group was 37 The family pattern of disease in adolescent colloid goitre is given in Table 6 Thirteen of the 52 relatives were one of parents of the patients. Out of 11 mothers with thyroid disease 3 had had adolescent goitre that had regressed spontaneously· 2 thyrotoxicosis; 1 auto-immune thyroiditis; 1 colloid goitre· and 4 not specified goitre In comparison, 15 mothers in the group auto-immune thyroiditis had thyroid disease· 2 thyrotoxicosis; 5 auto-immune thyroiditis; 5 colloid goitre (proved by biopsy)· and 3 thyroid enlargement of unproved etiology

Discussion

Diagnostic aspects

The present material consisted of the remainder of an originally unselected series of adolescent goitre from which certain types of goitre (Table 1) were excluded on the basis of observations made. Auto-immune thyroiditis was diagnosed on the basis of clinical immunological and cytological findings [4 10 17] Iodine deficiency was excluded on geographical grounds. The area is bordered on one side by a coast and goitre is not common in the population there Iodine deficiency was, however suspected in one case on the basis of the results of the examination of the iodine turnover (Case 7) Exogenous factors were noted in 3 cases but were judged as insufficient in 2 of them to be

TABLE 6 *Diseases in relatives of 37*[a] *adolescents with colloid goitre.*

Diagnosis	Total	Number of affected relatives				
		1st degree			Other relatives	
		Mothers	Fathers	Sibs	Maternal	Paternal
Thyroid disease						
Thyrotoxicosis	11 (8)[b]	1	—	—	5	5
Autoimmune thyroiditis	2 (1)	1	—	1	—	—
Thyroid adenoma	2 (2)	1	1	—	—	—
Adolescent goitre	4 (4)	3	—	—	—	1
Goitre unspecified	33 (17)	5	1	—	13	14
Total	52 (24)	11	2	1	18	20
Other diseases						
Diabetes mellitus	8 (6)	—	1	—	3	4
Rheumatoid arthritis or rheumatic pains	12 (10)	3	—	—	6	3
Allergic diseases	3 (1)	1	—	—	2	—
Organic disease of the central nervous system	1 (1)	1	—	—	—	—
Pernicious anaemia	1 (1)	—	—	—	1	—

One family history could not be obtained (Case 23) and two propositi were sibs (Case 28 and 29).

Figures in brackets represent number of propositi with affected relatives.

entirely responsible for the goitre (see material). The goitre was not believed to be due to overt defective synthesis because the goitres, except for the cystic ones, were not nodular and the patients were euthyroid.

A positive diagnosis of adolescent colloid goitre is based on the finding of mild to moderate enlargement of the thyroid gland with a smooth surface and often normal elastic consistence (see below) absence of increased PBI[131] in radioiodine tests and the absence of thyroid antibodies in the serum. Only in doubtful cases are aspiration biopsy and cytological examination necessary Such cases are those with low insignificant thyroid antibody titres. Thyroid antibodies occur in low titre in apparently normal persons without goitre the incidence increasing with age and often in the presence of thyroid disease in general [5]. The histological correlate of these antibodies consists of small focal usually non-progressive changes of the type seen in thyroiditis and which should be regarded as an accompaniment of an ageing or pathologically changed thyroid and of no practical clinical significance

The nature of the goitre

The goitres in the present material can with but few exceptions be regarded clinically as simple diffuse goitre occurring in puberty almost only in females and without any other symptoms of disturbed thyroid function. The goitres varied somewhat in size and in consistency but they were always homogeneous and elastic and had smooth, even surfaces. It was particularly the moderate to large goitres that felt as if they contained abundant colloid,

and in these the cytological picture confirmed the palpatory finding These cases thus justify the descriptive term of colloid goitre. On the other hand, it was not possible to judge the amount of colloid in the very smallest goitres. The cystic goitres differed markedly from the other goitres. Cystic changes need not be a manifestation of another type of thyroid disease but may be of non-specific degenerative nature.

Iodine studies

Clinical and laboratory studies showed that the patients were euthyroid. The uptake of radio-iodine by the thyroid was often highly normal or slightly elevated, while the content of PBI^{131} in plasma was within normal limits but with a number of low values. The PBI^{131} values did not indicate a somewhat increased turnover of iodine in the thyroid, as would be expected in *functional hyperplasia* The results could perhaps best be explained by the assumption that the tracer iodine was taken up in a normal way by the enlarged thyroid, where it was diluted in a large amount of colloid. The result may however also *per se* be compatible with a certain degree of iodine deficiency or with a slight enzymatic defect in the synthesis of the hormone

In 13 of 14 patients examined an increased difference was found between the content of protein-bound iodine and of butanol-extractable iodine indicating an increased content of nonbutanol-extractable iodoproteins in the plasma. Similar results have been reported in cases of atoxic goitre not related to puberty [7 0] A marked PBI BEI difference and hypothyroidism also characterize a form of familial inborn error of thyroid hormonogenesis [8]. Because of certain methodological problems it was difficult to evaluate the finding, but if the difference is true, it suggests a decreased capacity of thyroid to break down thyroglobulin (disturbed activity of the proteolytic enzyme?) or excessive production of abnormal iodoproteins in the thyroid or possibly an abnormal leakage of thyroglobulin or degradation products of it from the thyroid. The patients studied included both those with goitre in the family and patients without known goitres in the family

Aetiology and pathogenesis

The conception that goitre of puberty is a compensatory hyperplasia of a supposed increased hormonal requirement during puberty offers no explanation why only a few individuals, and nearly always girls, develop demonstrable goitre, and it *is not compatible with results of radio-iodine studies* or with the abundance of colloid found in most goitres The above conception may hold for a few very small goitres, but the idiopathic goitre of puberty should be regarded as an ordinary colloid goitre which develops under the influence of factors related to puberty

The familial occurrence of thyroid disease that cannot be explained by external environmental factors such as iodine deficiency suggests a genetic dispodtion to thyroid disease. The mechanism of such a disposition is still obscure and may be of more than one type The possibility of impaired ability to break down thyroglobulin has already been mentioned From a theoretical point of view there might be some congenital defect in the structure and/or function of the thyroid, the defect not manifesting itself in the form of goitre

until during periods of increased thyroxine requirement such as during puberty and pregnancy Variations in the severity and type of thyroid defects might help to explain why goitre sometimes occurs before or persists after puberty

Puberty doubtless plays an important role in the development of goitre but can probably precipitate goitre only in patients with some preexisting thyroid disorder The simplest way to explain the influence of puberty would be to assume that the requirements of thyroxine are increased during this period of rapid growth, but no evidence in support of this theory is as yet available. In children the thyroxine degradation rate is higher than in adults [11]. In females with adolescent goitre and increased iodine uptake Ingbar found an increased thyroxine degradation rate while girls with goitre and normal iodine uptake had a normal degradation rate [13] These results can be interpreted in two different ways. The hormone requirements during puberty may vary from one individual to another or they may be increased only during a certain phase of puberty In one of the present cases the rate at which the hormone was degradated was studied and found to be normal

The sex distribution and association of goitre with puberty and pregnancy suggest some kind of connection with ovarian function. The observation made by many that the goitre is larger during the last few days of the menstrual cycle point in the same direction. The effect of oestrogen and progesterone on the development of goitre has been studied in rats receiving propylthiouracil as a goitrogenic substance [8]. Oestrogen was found to curtail the development of goitre, while progesterone had the opposite effect. How the effect is mediated remains to be explained

Large doses of oestrogen will increase the plasma content of thyroxine-binding protein [6]. This, however only results in a transient increase of thyroid function, until a new equilibrium has been established. The PBI level is normal during puberty as is the thyroxine saturation of the thyroxine binding globulin (R Ordell unpublished results). Consequently this mechanism cannot play any role in the development of goitre

Therapeutic considerations

The definitive prognosis of adolescent colloid goitre in the present material can not yet be predicted, but it is obvious that goitre tends to decrease and often to disappear after puberty This together with the mildness of the condition suggests that it is advisable to leave the condition untreated during puberty On the other hand, treatment with thyroxine should be contemplated if the goitre is substantial and tends to persist after 18 to 20 years of age

Summary

Adolescent goitre is generally conceived as a special form of goitre induced by an increased hormone requirement during puberty But goitre at puberty is not a uniform condition. Some causes of goitre are known with certainty such as iodine deficiency and auto-immune thyroiditis. The present material of "idiopathic" adolescent goitre consisted of 39 patients (37 females, and males) aged 9 to 17 years. The patients were euthyroid and largely without symptoms except goitre The enlargement of the glands was usually small to moderate The glands were homogeneous

of elastic consistence and had a smooth, even surface Cytologically the goitres were characterized by active secretory follicular epithelium and abundant colloid. The uptake of radioiodine by the thyroid varied, but was usually near or just above the upper normal limit of the normal range The amount of radioactive hormone iodine in the plasma was, however not increased but if anything decreased.

Follow up was not long enough to allow any valid conclusions about the definitive prognosis of the condition. So far half of the goitres have decreased or disappeared but several show no signs of spontaneous regression.

A familial predisposition to thyroid disease was noted. The idiopathic adolescent goitre was not conceived in the present investigation as an enlargement of the gland due to compensatory hyperplasia but as a colloid goitre on the basis of unknown structural defect or functional disorder of the gland and under the influence of some obscure factor or factors released or activated at puberty

References

1 Clayton G W and Johnson C. M.: Struma lymphomatosa in children. Report of 12 cases. *J Pediat*, *57* 410, 1960

Clements, F W Natural history of endemic goitre in affected persons. In *Endemic Goitre*, WHO monograph series no. 44, p 235, Geneva, 1960,

3. De Groot L. J and Stanbury J B.: The syndrome of congenital goiter with butanol insoluble serum iodide. *Amer J Med* *27* 586, 1959.

4. Doniach, D Nilsson L. R. and Roitt I. M.: Auto-immune thyroiditis in children and adolescents. II. Immunological correlations and parent study *Acta Pædiat Scand*, *54* 260, 1965

5. Doniach, D and Roitt, I. M.: Clinical applications of thyroid autoantibody tests. In *Clinical Aspects of Immunology* Ed. Coombs and Gell, p. 611 Blackwell's, Oxford, 1963.

6. Dowling, J T Freinkel, N and Ingbar, S. H. Effect of diethylstilbestrol on the binding of thyroxine in serum. *J Clin Endocr* *16* 1491 1956

7 Dowling J T Ingbar, S. H., and Freinkel, N Abnormal iodoproteins in the blood of eumetabolic goitrous adults. *J Clin Endocr* *21* 1390 1961

8. Eakin, B A., Drathman M. B and Dewitt Pettit M.: The influence of ovarian hormones on goitrogenesis. *Endocrinology* *69* 195, 1961.

9. Gaskkerpan F S., Lowenstein J M., Spilker, P and Cramp S.: Abnormal iodoproteins in nontoxic goiter *New Engl J Med* *269* 830, 1963.

10. Gribetz, D Talbot N B and Crawford, J D Goiter due to lymphocytic thyroiditis (Hashimoto struma) Its occurrence in preadolescent and adolescent girls. *New Engl J Med*, *250* 555, 1954.

11. Haddad H. M.: Rates of I[131]-labeled thyroxine metabolism in euthyroid children. *J Clin Invest* *39* 1590, 1960.

12. Hubble, D Thyroid disorders in childhood. *Brit Med J* *I* 875, 1956.

13. Ingbar, S. H. Clinical and physiologic implications of thyroxine turnover in man. In *Clinical Endocrinology I* Ed. E. B. Astwood, p. 91 Grune & Stratton, New York, 1960.

14. Leboeuf G Bongiovanni, A. M., Steiker, D D and Eberlein W R.: Immunologic and thyroid function studies in euthyroid children with goiter *J Pediat* *58* 477 1961

15. Nilsson L. R., Borgfors, K Gamstorp L, Holst H. E., and Lidén G Nonendemic goitre and deafness. *Acta Pædiat Scand*, *53* 117 1964.

16. Nilsson L. R. and Doniach, D Autoimmune thyroiditis in children and adolescents I. Clinical studies. *Acta Pædiat Scand*, *53* 255, 1964.

17 Nilsson L. R. and Persson P S Cytological aspiration biopsy in adolescent goitre *Acta Pædiat Scand* *53* 333, 1964.

18. Norix T and Rosenberger B Unpublished results.

19. Saxena, K. M. and Crawford J D Juvenile lymphocytic thyroiditis. *Pediatrics* *30* 917 1962.

20. Skanse, B. and Hedenskog, I.: The determination of serum protein-bound iodine by alkali incineration. *Scand J Clin Lab Invest*, 7: 291, 1955.
21. Sterling, K., Lashof, J. C. and Man, E. B.: Disappearance from serum of I^{131}-labeled l-thyroxine and l-triiodothyronine in euthyroid subjects. *J Clin Invest* 33: 1031, 1954.
22. Wilkins, L.: *The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence*. C. C. Thomas, Springfield, Ill., 1957

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Suicidal Attempts Made by Children

by ULF OTTO

Introduction

In a previously published survey Bergstrand & Otto [5] have reported on a material covering 1 27 Swedish children and adolescents under 21 years who in the period 1955–1959 were treated because of suicidal attempts, in hospitals and other institutions in Sweden In that survey a series of external circumstances surrounding the suicidal acts were explored Taking this material as a point of departure Otto has made a more thorough analysis of suicidal acts, especially in respect of the presence of conditions of psychiatric illness and personality variables in the group investigated [13] the possible presence of a presuicidal syndrome [12] and suicidal acts committed by young men doing their military service [14], pregnant girls and women [16] and by school children [15] The purpose of the present survey is to give an account of the youngest group within the above material—with the latter as a point of departure—namely of children and adolescents under 14 years

Method of Selection

The principle followed in the collection of the material, and the method of selection have been explained previously [5, 13 14]. For the five-year period 1955–1959 the case notes have been collected and studied on patients under 21 years who have been treated because of suicidal attempts in *all* hospitals in Sweden to which cases of this nature can supposedly be referred This implies that a total of 471 hospitals and other institutions have been approached 458 ([illegible] %) of which have forwarded case notes, covering 1727 cases in all. The treatment of such a material must be uneven, and therefore only such data as sex, age and the character of the suicidal attempt have been recorded for the entire material. A selected portion, representing approximately 1/3 of the cases, have undergone psychiatric exploration. The distribution by sex and age of this portion shows a statistically satisfactory accordance with the entire material. On these more thoroughly penetrated cases more detailed data and psychological facts have been brought out, such as heredity conditions of growth and environment, past illnesses, personality as well as mental and somatic morbid conditions. The uneven treatment, which is naturally a weakness in a material of this nature is counterbalanced by the fact that it covers the entire country and that it is not limited to a restricted client is derived from a given district hospital department or hospital.

Summary of earlier results from own investigations

Of the 1727 patients included in the material 351 (20 %) are boys and 1376 (80 %) are girls. A higher incidence of suicidal at

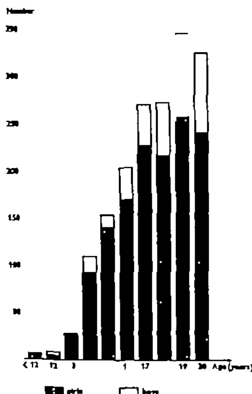


Fig 1 Distribution by age and sex of children and adolescents having attempted suicide (Bergstrand & Ott [5])

tempts ppears with increased age, and with special rapidity from the age of 13–14 years. An increase also took place during the period 1955–1959 Social group III is considerably overrepresented (82.5%). The reasons indicated for the suicidal ttempts showed the following distribution: Love problems 39.2%, home and parental problems 32.1%, school problems 6.2%, mental illness 17.5%, military service 1 7%, and pregnancy 3.4%.

Of the method recorded 86.9% had ingested narcotic drugs, while other methods, such as hanging and strangulation, shooting, gas poisoning, leaping from height drowning, cutting self etc. accounted for the remaining 13.1%.

Among conditions of psychiatric illness the neurotic group represent 53.0% (neurosis 23.2% and re . tic neurotic depressive 29.8%). The psychoses constitute 16.5% (schizophrenia 11.8%, and manic-depressive psychosis 4.7%) Primary or early character disorder (psychopathy) has been recorded in 13.2%, puberty insufficiency in 10.7%, and cerebrolesional conditions in 6.6%.

The personality variables observed were distributed as follows: hysteroid 35.7%, infantile 32.7%, oligophrenic 14.5%, asthenic 12.9%, schizoid 3.6% and cycloid 0 6%.

It has not been possible to determine any specific presuicidal syndrome. The most frequent change in personality and behaviour during the three months preceding the suicidal attempt was the appearance of depressive symptoms, 38.3%, followed by symptoms of the nature of anguish, anxiety sleep disturbances and psychosomatic symptoms, 30.1%. Less frequent are symptoms of the type irritability aggressiveness, labile affectivity 16.2%, signs of social maladjustment such as neglect of work of school and personal hygiene, vagrancy truancy increased alcohol consumption, promiscuity and other forms of asocial behaviour 12.1%, while symptoms indicative of psychosis, such as hallucinations, were observed in 3.2%.

Previ us Surveys

Suicidal acts in children and younger adolescents have earlier been the object of several surveys. Due t the restricted number of vailable cases in the younger ages, these surveys have however generally consisted of descriptions of individual cases or f small number of cases. Certain statistical computations have nevertheless, been made

An account will be given of a certain number of results of surveys made in different countries. Bakwin & Bakwin (2) have denoted that in Germany the number of suicides committed by children under 15 years, rose from a yearly average of 38.2 in the period 1869–1873 to 64.8 during the years 1894–1898. In Moscow 4.5 % of all registered suicides in the years 1908–1909 were committed by children in the age group 8–14 years. The corresponding figures for the

TABLE 1 *Mortality per 100 000 from suicide in selected countries 10 to 14 years by sex (WHO 1956)*

Country	Years	Males	Females
Germany Federal Republic	1952 1954	1.9	0.5
Portugal	194 –1949	1 7	0.3
Denmark	195_ 1954	1.3	0.2
New Zealand (without Maoris)	1950–1954	1.3	—
Finland	195_–1954	1.2	0.4
Switzerland	1951 1953	1 1	0.4
Japan	1953–1954	0.9	0.8
Chile	1950–1951	0.8	0.6
Sweden	1951 1952	0.7	0.1
United St tes	1951 1953	0.7	0._
Austria	1952 1954	0.7	0.3
France	1952 1954	0.6	0.2
Australia	1951 1953	0.6	—
Italy	1951 1953	0.6	0.2
Netherlands	1952 1954	0.5	—
Canada	1950–1954	0.5	0.2
Spain	1951 1953	0.5	0.2
Union of South Africa (European population only)	1951 1953	0.2	—
England and Wales	1952 1954	0._	0.1
Ireland	1952–1954	0._	—
Scotland	1952–1954	—	0.2
Norway	1952 1954	—	0.3

U.S.A. were 1934 0.22% for the age group 10–14 years, and 1.6% for adolescent of 15–19 years.

In the U.S.A 4 suicides were registered in 1923 the authors of which were children under 10 years. For children under 14 years the number [10] was 40 in 1926, in 1937 53, 1938 48, 1939 33, 1942 64, 1943 59 1944 46 and 1948 50

Jacobziner [9] studied all children in New York City of 6-19 years who had made suicidal attempt through the ingestion of some kind of poison Of 299 cases on which reports were available for a period slightly shorter than four years, 14 (5%) were 9–12 years, and 28 (9 %) were 13 years. In 1947 44 children in the age group 10–14 years committed suicid in the USA, according to Jacobziner

Table 1 (from Epidemiological and Vital Statistics Report, Mortality from Suicide" presented by WHO 1956 [21]) shows the frequency of suicides in the ages 10–14 years in different countries. Great caution must however be observed in making comparisons between the suicidal frequency in different countries. For religious and moral reasons such acts are subject to varying evaluation and consequently registered differently from country to country Hence there are naturally important differences between the national statistics in the distribution of suicidal acts.

The general observation concerning sex specific differences in relation to completed suicides applies to these younger ages too, namely that the boys predominate In the age group 10 14 years suicides are 2½ t mes more frequent among boys than among girls [21]. According to the official Swedish statistics, suicides per 100,000 inhab tant in the period 1951–1955 were comm tted by

0.62 men and 0.16 women in the age group 10–14 years, i.e. for both sexes 0.39

Hartelius [8] has found that the youngest authors of the suicides registered officially during the period 1925–1950, namely those of 11 and 12 years, were solely boys, whereas among seven adolescent of 13 years four were girls. Hartelius also found that of the authors of suicides committed in Sweden in the period 1925–1937 boys under 15 years represented 0.2%, and girls 0 1% of the total number whereas the distribution by sex for the period 1938–1950 was the reverse i.e the boys represented 0 1% and the girls 0.2%.

Mulcock [11] found that out of 114 suicides committed in England by children of 10–15 years, 112 were boys, and only 2 were girls.

Dublin & Bunzel [6] also found a preponderance of boys over girls among suicides under 15 years.

Baer [1] considered that the foremost reasons for suicidal ets committed by children were shame, remorse and pangs of conscience which were recorded in approximately one third of 336 children in the age group 10–15 years, who had committed suicidal acts in Germany during the period 1884–1893, and whose cases he had studied. Mental illness was considered to be the cause in less than 10%. Among other causes denoted were: anger and rows (11 4%), physical pain (2%), depravity (1.5 %), and anxiety (1%) N tangible cause emerged in 31.8% of the cases.

Tramer [20] has reported on one 11 year old boy and two boys of 12 years, who attempted suicide He distinguished between the following three conceptions: (1) Readiness t commit suicide, (2) Readiness t envisage suicide almost reaching readiness to commit it, and (3) Readiness t envisage suicide without being at all ready t commit t.

Bender [4] found among the six hildren of six to nine years, of whom she made a follow-up examination when they were 13–17 years old, that the most frequent method used was to jump out of the window whereas adolescents, the older they are the more often they turn to ingestion of poison. The cases described by Redlich & Lazar [17] have likewise as a rule, used so-called active methods, as did the nine-year-old boy described by Baer and two of the three children described by Harding [7] (see under Casuistry)

Casuistry

From the isolated casuistic information published the following may be related.

Redlich & Lazar described a *three year old boy* who was found unconscious together with his 1½-year old sister in a gas-filled room where the gas cock was open. The children were rescued with considerable difficulty The boy explained that he wanted to kill himself and his sister because their mother had not taken them with her for a walk. In the presence of his children the boy father had earlier in the day told about a man who had attempted to commit suicide by gas-poisoning. The same writers have described a *f re year-old girl* who had jumped out f the window and died from her injuries. She lived in an environment where she was badly treated. A *6½-year-old boy* jumped int a river and was drowned, after being scolded by hi mother Previously he had repeatedly threatened with suicide The autopsy revealed unmistakable brain alterations. An *8½ year-old boy* was caught by his mother when he pilfered money from her When the father returned in the evening, the boy jumped out of the window from the third floor but only received minor injuries.

Baer has described a *nine-year-old boy* who had been scolded by his mother and punished by his f ther H jumped out of the window from the fourth floor and died.

Harding described three children in Sweden who had attempted suicide A *te year-old boy* who had for a long time been ridiculed by his parents because of his enuresis and encopresis, repeatedly tried t hang himself A *seven-year-old boy* with a distinct hereditary disposition for manic-depressive psychosis, tried t drown himself after an

TABLE 2. *Distribution by age and sex among children under 14 years who have made suicidal attempts*

Age	Number	Sex ♂	♀
10	2	2	0
11	6	2	4
12	9	3	6
13	25	2	23
Total	42	9 (21.4 %)	33 (78.6 %)

outburst of anger directed towards him by his sick mother. An *eight-year-old girl*, affected by compulsory neurosis, who later developed schizophrenia, tried to commit suicide by refusing to eat and drink probably from a desire to take vengeance, as the mother had repulsed her for several years.

Results

With the arrival of the age of 14 years there is a rapid increase in the frequency of suicidal attempts.

Table * shows that an increase occurs from 2 registered cases among children about 10 years old to 6 cases of about 11 years, 9 about 12 years, and 25 about 13 years. Thus the markedly accelerated increase, coinciding with and including the puberty period has begun.

The youngest case is a ten year-old boy-

15 year-old boy the eldest of two siblings whose parents have been divorced for six years. The patient is living with his mother. He is described as emotionally unstable and has at an earlier age reacted to adversities by convulsions provoked by affect. He has pilfered money both at home and in stores. Because of the boy's violent grief on the occasion of his grandmother's recent death, to which he reacted *inter alia* with sleep disturbances, the doctor had given him 20 phenemal tablets. After a row with the mother and in an outburst of affect, the boy ingests at least 15 tablets and is brought to the hospital in a st te of unconsciousness.

In the age group of children under 14 years the girls represent 78.6 % and the boys 21.4 %. This sex distribution corresponds to that of the entire material, where the girls constitute 80 %, and the boys 20 %. Thus the preponderance of the girls among the authors of suicidal attempts also applies to the prepuberty years. But it is, nevertheless not so obvious before the age of 13 (7 boys and 10 girls).

Table 3 shows the causes recorded of the suicidal attempts made by the youngest group compared with the distribution of such causes in the entire material. Home and parental problems were indicated in 69.0 %, school problems in 16.7 % and love problems in 14.3 %. For the large material love problems were recorded

TABLE 3. *Reasons for suicidal attempts by the young group compared with the rest of the material.*

Reason	Young group N	%	Entire material N	%
Home and parental problems	29	69.0	292	30.4
Love problems	6	14.3	388	40.3
School problems	7	16.7	45	4.7
Others	—	—	226	23.6
Total	42	100.0	[illegible]	100.0

the cause in 40.3 %, home and parental blems in 30 4 % and school problems in 5.7 %. The difference between the two aterials is highly significant (**)

Passive methods of the suicidal attempt ... represented by 81 % among the ·oung group and the ingestion of tablets 78 %. In the age group 10–1. years 14 .e 82 % have ingested sleeping tablets. Comparison with the entire material shows concordance since 86.9 % of the latter material had ingested tablets, while 13.1 % were distributed among other methods.

A more thorough analysis of the mental state of the group is not possible as a satisfactory psychiatric exploration in dicating the diagnosis was only under taken in a few cases

Discussion

A series of earlier surveys shows that suicidal acts are comparatively rare prior to the onset of the puberty period, which in itself cannot be definitely delimited in terms of age. We have however pro- visionally found (Bergstrand & Otto [5]) that from the age of 13–14 years a rapid rise occurs in the frequency of suicidal at tempts. The results reported in this survey cover the years from 13 downwards not with a view to setting a definitive limit t that age, but in order to take this group as a point of departure for stating and discussing certain conditions parti cular to the younger ages. The youngest cases are two 10-year-old boys. A sharply accelerated increase occurs from the age of 13. In connection with this rise a dif ferentiation between the sexes emerges which is not as tangible however in th age group 10–12 years where the girls constitute 59 (58.8) % and the boys 41 (41.2) %, so that the same sex distribution prevails as was recorded for the entire material, namely 80 and *0 per cent res- pectively

Hartelius has found, among completed suicides in the age group 11–12 years in the period 1925–1950 that only boys were registered, whereas among the 13-year old children slightly more than 50 % (4 of 7) were girls. In the younger ages there was a less marked predominance of boys as regards completed suicides, compared to the situation in older ages. On the basis of Hartelius observations and of the ma terial discussed in the present survey the sex-specific differences characteristic of older adolescents and adults both as regards completed suicides (male pre- ponderance) and suicidal attempts (female preponderance) do not seem to be present in the younger ages.

The difference between causes recorded for the youngest group and for the rest of the entire material is tangible In the rest of the entire material love problems were the cause in 40.3 % and home and parental problems in 30 4 %, whereas school problems constituted a minor part 5 7 % For the youngest group entirely different figures come to light indicative of the fact that the dependence on the home and parental situation is more pro- nounced, the lower the age of the child. Home and parental problems dominate with 69 0 %. Love problems, in the sense of problems connected with the contact with the opposite sex, constitute na turally enough a minor part namely 14.3 %. On the other hand school pro- blems represent a larger part 16 7 %, than

in the entire material. This corresponds to the conception of Harding that the most essential motive probably is the conviction that those who should give the child security namely the parents have failed to do so Bender has found that children, who commit suicidal acts have home and family situations which often reveal noteworthy conditions. The cases described by Redlich & Lazar and by Baer are also pointing towards the con clusion that problems inherent in the home situation constituted the dominant and also the provoking factor in the younger ages.

Two marked strains in the home situation are demonstrated in the following case

12 year-old boy the eldest of three half siblings; his biological father is a Danish warehouse worker having abandoned the boy mother who remarried later after the birth of the patient. During his years of growth the patient lived with various foster parents and in different children's homes, but has now lived with the biological mother for some years. He is unable t get along with his stepfather who takes out his bad temper on the patient and f vours his own children at the patient expense. The latter reacts by showing nvy and jealousy H ingests about ten sleeping t blets and is brought to the hospital unconscious. Upon wakening up, he utters regrets because he did not take more t blets. He says that he is unhappy in his home and intends to drown himself H is diagnosed as a slightly depressed, hysteroid boy of normal intelligence (IQ = 114) with pronounced destruct ve tendencies.

The next example is a girl, for whom the school situation constituted the provoking factor but where a series of noteworthy conditions emerge when the case is penetrated.

11 year-old girl, the eldest of two siblings. The father is an emotionally unstable ex acting and dominating academician, while the mother is described as singular egocentric and callous. The girl sabotages the home life through her provocative behaviour She is attending a girls school neglects her schoolwork and often plays truant. She has earlier had convulsions provoked by affect and psychomotor fits. Earlier examinations have revealed that the patient has a pathologic EEG (without any specific focal find ings, however). She is aggressive emotionally unstable, dominating, with a pronounced need of self-assertion In connection with an acute conflict situation in school she expresses wariness of life, ingests an unknown number of sleeping tablets, whereupon she is brought to the hospital, unconscious. She is diagnosed as an aggressive anguished, intelligent girl (IQ according to Terman Merrill = 156) with severe emotional and social problems.

Other causes have been indicated by Schneidman & Farberow [16] such as dread of punishment anguish, shame, remorse and pangs of conscience conditions and reactions which may well agree with the causes emerging from our ma terial but which are of such universal validity that their respective appearances are difficult to record.

Bender as well as Redlich & Lazar have found that active methods are the most frequent in the youngest group This does not correspond to the findings in this material, where the frequency of passive and active methods, respectively is in keeping with the entire group namely showing a substantial preponderance of passive methods.

Bender considers that the more apparent the aggressive component the more the children recur to violent methods Like Schilder & Wechsler [19] Bender

desires to emphasize that children have a conception of death which differs from that of adults. Younger children have a more realistic and matter-of fact attitude towards death. They do not believe that they themselves are going to die: death is a matter for old people. For them death is associated with the idea of violence. Very young children conceive of death as a reversible condition. Hence they often speak of death in an indifferent manner which is shocking to adults. The child reacts through an attempt to escape in a tense situation which is usually due to deprivation of love and causes aggressive tendencies which, under the impact of feelings of guilt turn towards the child itself. Here we find a reasoning similar to that stated by Harding. The suicidal attempt is at the same time a means of punishing the environment and of obtaining more love.

A more thorough analysis of the mental state of the group is impossible as the diagnosis has been recorded only in a few cases. Bender among others has, however emphasized that children, who commit suicidal acts, often show deviating personality traits. In lieu of such analysis certain elucidating cases will be described in the following.

Prepsychotic syndrome in an 11 year old boy:

11-year-old boy who during his stay in a child psychiatric hospital department for exploration makes several suicidal attempts by means of trying to touch electric wires, jumping out of the window, leaping from fire-ladder and injuring himself with knife. He is diagnosed as sufferering from a prepsychotic condition with symptoms of anguish and clouding of consciousness, and shows signs of organic brain injury.

12 year-old feeble girl living under the pressure of too heavy demands

12-year-old girl, the eldest of three siblings, whose father is a pedantic, bitter and exacting businessman, while the mother who is a housewife, is described as very critical and of a distinctly (x)old disposition. The girl's somatic development has been retarded, and she has had considerable difficulties in coping with her schoolwork. In addition she has contact difficulties with her schoolmates. She has pilfered money in order to satisfy her strong craving for sweets. Gradually she has become increasingly listless, passive, has lately begun to play truant, reads only weeklies and is glued to the TV. In connection with a conflict with her best friend, she expresses suicidal ideas, and a few days later ingests an unknown number of so-called headache tablets. She is brought to the hospital in a state of intoxication, is returned to her home, but is readmitted to the hospital a few days later having ingested sixteen tablets. The psychiatric examination shows that she is a feeble (IQ = 83) isolated girl, who is more depressive than hysteroid.

Signs of increasing insufficiency and, as the provoking factor, moments of frustration in school are demonstrated in the following case:

13 year-old girl, the youngest of four siblings. The father died about ten years ago, and the mother is earning her living as a barmaid. For the last six months the patient has been out of balance, nervous, restless and in constant conflict with her mother. She cries frequently, has been supposed to suffer from over-exertion and has been treated for anemia. When her teacher informs her that she will not be up to the pass standard in a certain subject at the end of the school year, she reacts violently and ingests 20–30 sleeping tablets. She is brought to the hospital, unconscious. Nothing tangibly noteworthy is revealed in the psychiatric examination.

Summary

Of a series covering 1727 children and adolescents under 21 years, from the whole of Sweden, who during the five-year period 1955–1959 had attempted suicide the 42 cases who were under 14 years have been explored more thoroughly The youngest case is a 10-year-old boy A more even sex distribution prevails in the younger ages contrary to what is true of the older ages, where there is a marked preponderance of girls. In the large material love problems, followed by home and parental problems are the primary causes. Among the younger children home and parental problems, followed by school problems, are the most common causes. Contrary to the findings of other writers, passive methods predominate in the present material among the younger clientele who attempt suicide

References

1 Baer, A *Der Selbstmord im kindlichen Lebensalter* Leipzig, 1901
2 Bakwin H.: Suicide in children and adolescents. *J Pediat, 50* 749 1957
3. Bakwin H and Bakwin R. *Clinical Management of Behavior Disorders in Children.* W B. Saunders, Philadelphia and London, 1960
4 Bender, L. *Aggression, Hostility and Anxiety in Children.* Charles C. Thomas, Springfield, Ill. 1953.
5. Bergstrand C. G and Otto U Suicidal attempts in adolescence and childhood. *Acta Pediat Scand, 51* 17–26, 1962.
6 Dublin L. I and Bunzel, B. C.: *To Be or Not to Be" A Study of Suicide* New York, Smith and Haas, 1933
7 Harding G Diskussion kring suicidalproblemet i barna- och ungdomsåren. *Nord Med 59* 330, 1958.
8. Hartelius, H. Suicide in Sweden. *Acta Psychiat Neurol Scand, 32* 151 1957
9 Jacobziner, H.: Attempted suicides in children. *J Pediat 56* 519 1960
10 Kanner, L.: *Child Psychiatry* Charles C. Thomas, Springfield, Ill. 1960
11 Mulock, D Juvenile suicide *Med Officer 94* 155, 1955.
12. Otto U Changes in the Beha iour f Children and Adolescents preceding Suicidal Attempts. *Acta P ychiat Scand, 40* 386, 1964
13. — Suicidal Attempts in Adolescence and Childhood—States of Mental Illness and Personality Variables. *Acta Paedopsychiat, 31* 397 1964
14 — Suicidal attempts made during compulsory service. *Acta Psychiat Scand 39* 298, 1963.
15 — Suicidal attempts mad by hildren and adolescents because of school problems. *Acta Pediat Scand, 54* 348, 1965.
16. — Suicidal attempts made by pregnant women under 21 years. *Acta Paedopsychiat, 32* 276, 1965.
17 Redlich, E and Lazar, E.: *Kindliche Selbstmörder* Springer Berlin, 1914
18 Shneidman E. S. and Farberow N L.: Clues to suicide. *Pub Health Rep, 71* 109, 1956.
19 Schilder, P and Wechsler, D.: The attitude of children toward death *J Genet Psychol, 45* 406, 1934.
20 Tramer, M.: Zur Frage des Selbstmordversuches und Lebensüberdrusses im Kindesalter *Acta Paedopsychiat, 4* 109 1949
21 W H O Mortality from suicide *Epidemiological & Vital Statistics Report, 9* 243, 1956.

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Measles Vaccination

V. The Booster Effect of Purified Hemagglutinin in Children Previously Immunized with this Product or Formalin Killed Vaccine

by E. NORRBY, R. LAGERCRANTZ and S. GARD

In previous studies the immunizing effect of purified hemagglutinin (HA), the TE (Tween-ether) vaccine, both in primary immunization [7] and for revaccination [6] was analyzed. For comparison groups of children immunized under identical conditions with a formalin-killed (FK) commercial adjuvant vaccine were included in the trials. The TE vaccine contained no adjuvant and was 3 to 4 times more potent than the FK vaccine as determined in guinea pig potency tests. No difference in serological responses was detectable between the two groups of seronegative children given three monthly doses of the two vaccines [7]. However, when used for revaccination of children previously vaccinated with FK vaccine [3] the increase in serum titer was considerably higher among children given TE than FK vaccine [6]. Final titers reached were higher than those recorded after natural measles.

The present report describes the results in further clinical and serological follow up analysis of the groups of children given their primary vaccination with either one of the two vaccines [7]. The responses to administration of TE vaccine of moderate potency in both groups of vaccinees 17 months after the primary immunization was also analyzed.

Supported by grants from the Swedish Medical Research Council (Project No. Y 213).

Material and Methods

Vaccine preparation. An experimental batch of TE vaccine prepared partly according to previously described methods [7], but purified by use of a two-step procedure including (a) separation in a two-phase system containing polyethylene glycol (Carbowax 6000) and dextrane sulphat (limiting viscosity nr 70 ml/g) and (b) adsorbtion-elution from ZnOH. The purified HA was suspended in phosphat buffered physiological saline pH 7.2, and no adjuvant was added. The vaccine preparation was diluted to a guinea pig potency value 6 to 8 times lower than that of previous vaccine batches [7]. It had an HA activity of 20.000 HA units per ml and the protein content was 0.1 mg per ml.

Study population. Two groups of children vaccinated at the age of six months to two years with either FK or TE vaccine [7] were 17 months thereafter inquired about measles exposure, bled and given 1 ml of TE vaccine subcutaneously. Ten to 14 days later a second finger tip blood sample was collected.

Serological analyses. After removal of the

erythrocytes by centrifugation of the heparinized blood samples and inactivation at 56°C for 30 minutes the concentration of measles HI antibodies was determined by the technique previously described in detail [3] All sera were tested simultaneously The titers given refer to final dilutions after addition of antigen. The lowest dilutions tested were 1 10 in the pre-booster and 1:40 in the post-booster samples. A human measles convalescent serum was used for standardization of the HA antigen and was also included in the tests as a reference to allow comparison with previous titrations.

In some children, who were expected to exhibit high titers, a venous pre- and post booster bleeding was made in addition No anti-coagulant was added The samples were used for testing of the reliability of the finger tip blood sampling technique and fo determination of their content of 19S and 7S antibodies. It was confirmed that identical HI titers were recorded in sera collected from one and the same vaccinee by the two techniques of bleeding

Separation of 19S and 7S antibodies. The technique used by Svehag & Mandel [10] was applied. A serum sample diluted 1 2 in physiological saline was layered in a volume of 0.2 ml onto 4.8 ml of a 10 to 37 % linear sucrose gradient. After centrifugation in a swinging bucket rotor (SW39 Spinco) at 35 000 rpm for 20 hours under rigidly refrigerated conditions 20 to 25 fractions of equal volume were collected from the bottom of the tube Some serum samples were run in mixture with rabbit reference sera containing 19S and 7S antibodies against ECHO virus type 7

Results

Correlation of HI antibody titers 11 and 17 months after vaccination The number of children available for bleeding both 11 and 17 months after vaccination were 33 and 31 in groups given TE and FK vaccine respectively Fig 1 illustrates the correlation between HI titers in sera from

Fig. 1 The correlation between 11 and 17 months post-vaccination HI titers in children immunized with TE (□) or FK (○) vaccine (△) denotes three children immunized with FK vaccine, who had been exposed to measles. The line of short dashes gives the zone of equivalence.

the two bleedings. No significant differences were detectable. The ratio of 17 to 11 months postvaccination geometric mean HI titers were 1.24 for the group of children given TE vaccine and 1 03 for the group given FK vaccine. The degree of deviation of the former value from 1 0 is within the limits of the accuracy of the tests. The mean titer in the group of children given FK vaccine was slightly higher than in the group given TE vaccine The ratios 11 and 17 months after vaccination were 1.55 and 1.24 respectively

The effects of clearcut exposure to natural measles A total number of 8 exposures to measles were recorded during a period of 17 months after the primary vaccination. Table 1 gives a summary of the exposed vaccinees and their reactions. Two of them who had received TE vaccine were exposed only a short time after vaccination. No clinical or serological reactions were

TABLE 1 *Clinical and serological data of children in connection with exposure to natural measles during 17 months after vaccination*

Child no. Type of vaccine	Time of exposure in months after vaccination	Incubation period in days	Temperature: Maximum C°	Temperature: Duration in days	Rash	Other clinical symptoms	HI serum titer[a]: Before	HI serum titer[a]: After
77 TE	2	—	—	—	—	—	80	80
113 TE	1	—	—	—	—	—	320	320
15 FK	13	11	37 7	1	0	Slight cough and coryza	40	80
22 FK	8	—[b]	39.5	2	Faint	None	640	640
27 FK	13	10	39.0	5	0	Slight cough and coryza	80	80
64 FK	11	11	40.3	4	Faint[c]	Slight cough and conjunctivitis	80	5120
85 FK	11	—	40.0	6	Faint[c]	None	80	2560
98 FK	10	10	39.3	3	0	Slight cough	320	40960

[a] Serological examinations were made 2 weeks, 11 and 17 months after vaccination. The pre-exposure sera of no. 77 and 113, however were taken at the injection of the third dose of vaccine.
[b] Source of exposure not known. A sister of no. 85 ran course of natural measles in parallel.
[c] The rash was located mainly to the feet.

detectable The remaining six children had been vaccinated with FK vaccine. All of them reacted, but most cases were mild. They ran a moderate fever of short duration and exhibited some catarrhal symptoms. It is noticeable that rash was either faint or absent The reactions were confirmed serologically to have been measles in three out of the six cases. One more child, Case 22 was also suggested serologically to have contracted measles, as was discussed in a previous paper [7] The remaining two children reacted with very mild symptoms and no rash after an incubation period of normal length 10–11 days. The reactions may have been mild measles but due to the lack of serological confirmation the definitive diagnosis has to remain unsettled. Both cases responded in the same way as unexposed children to a booster with TE vaccine (Fig)

Clinical nd serological reactions to a booster injection with TE vaccine 17 months after the primary vaccination No clinical reactions were recorded in connection with the administration of the booster dose

As in previous study [6] impressive secondary responses were evoked although the TE vaccine administered had a markedly lower potency than the batch of vaccine previously used. A comparison of pre- and post booster HI titers are given in Fig 2 As is obvious from this figure the increase in titers in general was higher among children previously vaccinated with FK vaccine than among those who had received TE vaccine The order of magnitude of increase in geometric mean titers were 82 6 and 20 0 times in the two groups, respectively The post booster geometric mean titers were 1 1*00 in the group of children given TE vaccine as primary vaccination and 1 5650 in children given FK vaccine. The difference in relative increases in titers of individual sera can also be seen in Fig 3 where cumulative percent

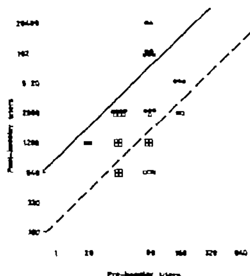


Fig. 2. The correlation between HI serum titers just before and two weeks after the injection of TE vaccine to children previously immunized with TE (□) or FK (●) vaccine. (Δ) denotes children exposed to measles. The line of short dashes and the full line have slope ~1 and passes through the points representing geometric mean titers in the groups of children primarily given TE and FK vaccine, respectively

ages of titer increases of various dimensions are illustrated. A comparative statistical analysis of the titer increase in the two groups of sera indicated a difference greater than expected by chance alone ($0.025 > p > 0.01$)

The molecular type of antibodies present in pre- and post-booster sera. Only 7S antibodies were demonstrable in pre-booster sera. In some of the sera tested the possible concentration of 19S antibodies must have been less than 3 % of the total amount of HI antibody when the sensitivity of the techniques are considered. The post booster sera contained a small fraction, 1–3 % of 19S antibody as exemplified by the serum in Fig 4 The picture, however was dominated by 7S antibodies, as should be expected from a secondary response [1 10]

Discussion

The present observations confirm and add to experiences gained from previous studies on the effect of inactivated measles vaccines [5 6]. Thus measles HI antibody titers reached after three monthly doses of vaccine decline 6 to 12 fold over a period of 8 to 11 months [5 7] After this a stabilization of the titer level occurs and no virtual change in titers takes place over a long period of time However in spite of the persistent antibody level and the state of sensitization massive exposure to measles in many cases leads to the appearance of mild clinical symptoms, usually without a typical rash. Evidently a titer of the order of 1 80 prior to exposure does not provide complete protection in some cases (Table 1) This prompts the question if there might be a difference in the relative effectiveness of antibodies appearing after natural measles and after vaccination with inactivated vaccine?

It is well established that a 12 % solution of gammaglobulin in a dose of about

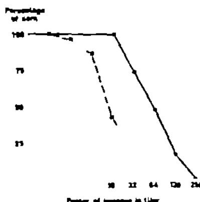


Fig. 3. Curves illustrating cumulative percentages of sera from children primarily immunized with TE (□ – – □) and FK (●——●) vaccine xhibiting various increases in HI titers after booster injection of TE vaccine.

0.06 ml/kg bw is sufficient to meliorate the clinical manifestations of measles when given within five days after exposure [9]. *An about six times higher dose in most cases* gives complete protection. Provided a gammaglobulin titer of 1 4000 to 1 8000 which is usually found with the HI test used in the present study [4], the doses just mentioned should give HI serum titers of only about 1 1 and 1 6, respectively. This might indicate differences in protective capacity of antibodies produced *in response to various types of immunization*. In this connection it should be remembered, however that the avidity of antibodies increases with time after immunization [2]. Therefore the plain titer values might not necessarily be a measure of the protective effect. This question can not be settled on the basis of present evidence. In any event it should be stressed that immunization with *inactivated* vaccine alone by a suitable schedule of administration will produce HI titers of long durability on a level that would seem to ensure complete protection [6].

The differences in secondary responses after administration of one and the same dose of TE vaccine to children primarily vaccinated with FK and TE vaccine is interesting. Although the potency of the TE vaccine used for primary immunization was *3 to 4 times higher* than that of the FK vaccine approximately similar results were obtained [7]. Mean titers at the time of the booster inoculation were also almost the same and yet the FK group gave a significantly better response. Apparently this group had maintained more active state of sensitization. Whether this is attributable to the presence of an adjuvant in the FK vaccine cannot as yet be

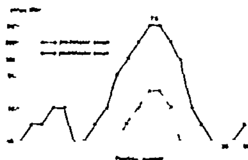


Fig. 4. The occurrence of 19S and 7S HI antibodies in sera from a vaccinee before (● – – ●) and after (○——○) the injection of booster dose of TE vaccine. The antibodies were separated by centrifugation in 10 t 37 sucrose gradient at 35,000 rpm for 20 hours under refrigerated conditions.

definitely concluded, although it appears probable. If so, inclusion of some adjuvant in vaccine intended for primary immunization will have to be taken into serious consideration.

On the other hand, the use of *adjuvants* in connection with *booster injections* would not seem to serve any useful purpose. In a previous study [6] children primarily vaccinated with three monthly doses of FK vaccine and boostered 22 months later with the same vaccine exhibited a 35-fold increase in geometric mean titer. In the present study children preimmunized with the same type of vaccine and boostered 17 months later with a TE vaccine of a potency *less than half* that of the FK vaccine exhibited an 83-fold rise in titer. This suggests that an adjuvant free vaccine might be relatively more effective in evoking secondary responses.

It has been described [8] that after a booster injection of measles antigen only 7S antibodies can be detected. However in systems where sensitive serological techniques were applied antibodies appearing

in a secondary response, although primarily of the 7S type were also shown to contain a small fraction of 19S antibodies in a quantity corresponding to the dose of antigen administered [1 10] The same type of composite secondary antibody response was also found in post-booster sera analyzed in the present study

Summary

Two groups of children immunized at the age of 6 months to two years with three monthly doses of either formalin killed (FK) or Tween-ether (TE) measles vaccine were submitted to clinical and serological follow up analysis up till 17 months after vaccination and then given a booster of TE vaccine of moderate potency

Among 8 cases of clearcut exposure to measles before the booster 6 responded with mild clinical symtoms, 3 without and 3 with a faint rash. An increase in HI serum titer was recorded in 3 out of the 6 cases. All of them had received FK vaccine.

Between 11 and 17 months after vaccination no change in HI titers was demonstrable. After the booster the geometric mean HI titer increased 20 times in children primarily vaccinated with TE vaccine and 83 times in children given FK vaccine In the pre-booster serum samples only 7S antibodies were detectable whereas the post booster serum samples in addition to 7S antibodies contained 1 to 3 % 19S antibodies.

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References

1 B uer, D C., Mathies, M. J and Stavitsky A. B. Sequences of synthesis of γ-1 macroglobulin och γ 2 globulin antibodies during primary and secondary responses to proteins, Salmonella antigens and phage. *J Exp Med*, *117* 889 1963
2. Jerne, N K.: A study of avidity *Acta Path Microbiol Scand, Suppl*, *87* 1951
3 Norrby E. Lagercrantz, R., Gard S. and Carlström, Gun Measles vaccination. I. Serologic responses t an inactivated vaccine *Arch Ges Virusforsch*, *13* 548, 1963.
4. Norrby E.: *Studies on Measles Virus*. Habilitation thesis. Tryckeri Balder Stockholm, Sweden, 1964.
5. Norrby E. Carlström, Gun Lagercrantz, R. and Gard S.: Measles vaccination. II. Antibody titers 6 to 9 months after vaccination with an inactivated vaccine. *Acta Paediat Scand*, *53* 533, 1964.
6. Norrby E., Lagercrantz, R. and Gard S.: Measles vaccination. IV Responses t two different types of preparations given as a fourth dose of vaccine. *Brit Med J I* 813, 1965.
7 Norrby E., Lagercrantz, R., Gard, S. and Carlström, Gun: Measles vaccination. III Serologic responses t immunization with purified hemagglutinin. *Acta Pædiat Scand*, In press 1965.
8. Schluederberg A. E. Modification of immune response by previous experience with measles. *Arch Ges Virusforsch 16* 347 1965
9 Stokes, J Jr., Maris, E. P and Gellis, S. S.: Chemical, linical and immunological studies on the products of human plasma fractionation. XI. The use of concentrated normal human serum gammaglobulin (human immune serum globulin) in the prophylaxis and treatment of measles. *J Clin Invest*, *23*. 351 1944
10 Svehag, S. E. and Mandel, B.: The formation and properties of poliovirus-neutralizing antibody II. 19S and 7S antibody formation: Differences in antigen dose requirement for sustained synthesis, anamnesis and sensitivity t X-irradiation. *J Exp Med* *119* 21, 1964.

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Chromatographic Pattern of Serum and Urinary Amino Acids in Cretinism

by MAMDOOH GABR, ZENAB ABD EL-GAWAD and FOUAD EL-BEHAIRY

In spite of the known effects of thyroid hormone on protein metabolism [4 6 10 12, 16, 17], there are only few references on the changes in the amino-acid pool in thyroid disorders. Hyperthyroidism is reported to cause an increase in serum amino-acid level both in adults [3] and children [2]. There are no reports on serum amino-acid pattern in congenital hypothyroidism. Sonoda [13] reported that leucine and arginine appeared in urine of cases of myxedema. The present study reports the changes in serum and urinary amino-acids in cretinism before and after adequate thyroid therapy

Material and Method

Twenty three cases of congenital hypothyroidism aged 2–14 years were studied. They included 11 boys and 12 girls. Diagnosis of hypothyroidism was est blished on clinical data as well as estimation of serum P.B.I 24 hr I[131] uptake, scanning of the neck following I[131] administration and radiological bone age Cases included 6 patients with goitrous cretinism, L patients with athyrotic cretinism and 3 patients with thyroid dysgenesis. Cases were classified as mild or severe according to the clinical features and delay in osseous development. None of the cases received any thyroid medication prior to examination. The patients diet was standardized on a protein intake of 2–3 g/kg/day for at least weeks before study Fasting blood samples were drawn for semi-quantitative estimation of serum amino-acids from all cases. Twenty-four hours urine was collected for studying the amino-acid pattern of the urine Serum and urinary amino-acid patterns were re-examined in 12 patients 2 3 months following adequat thyroid therapy on a dose of 60–90 mg dessicated thyroid/square meter surface area [15].

Ten normal children of the same age groups were studied as controls. There was no evidence of present or past history f disease known to affect thyroid function and/or amino-acid pattern in controls.

Technique

Serum samples were deproteinized and concentrated. Uni and bidimensional chromatograms were carried out on sheets of Whatman No. 1 filter paper Ascending uni dimensional chromatography was carried out using butanol, acetic acid and w ter as solvent and stained by 0.2% nin hydrin in acetone [7]. The stained chromatograms were scanned in the spinco analytrol unit This method was useful t give rapid qualit tive pattern of the serum amino acids and good separation was obtained in most cases. Bidimensional chromatograms were carried out using the miscible solvent of phenol, w ter ammonia as the first solvent N

TABLE 1 *Serum amino-acid scores in untreated cases of cretinism.*

No.	Age (yr)	Sex	Type of cretinism	Severity	Leucine	P.Alanine	Valine	Tyrosine	Alanine	Glutamic acid	Glycine	Aspartic acid	Arginine and lysine
					Scores of amino-acids in serum chromatogram								
1	4	M	Athyreotic		5	3	3		5	3	5	4	6
2	5	F	Athyreotic		3	3	3		5	4	5	4	3
3	4	F	Athyreotic		3	—	2		4	4	3	3	3
4	5	M	Athyreotic		4	2	3		5	4	6	4	1
5	3	M	Athyreotic		3	3	3		8	6	4	4	4
6	10/12	F	Dysgenesis		4	3	3		4	4	5	4	3
7	12	F	Goitrous		4	3	3		5	4	4	4	3
8	9	M	Goitrous		1	—	4		3	3	3	3	3
9	11	M	Goitrous		3	—	3		4	2	5	4	3
10	1	F	Athyreotic	Severe	2	—	2	Trace	4	3	4	3	2
11	2	F	Athyreotic		2	—	2		3	4	4	3	3
12	3	F	Athyreotic		3	—	3		4	3	3	3	3
13	4	M	Dysgenesis		3	—	2		3	3	3	4	3
14	3	F	Athyreotic		1	—	1		4	3	4	3	2
15	1	M	Athyreotic		5	4	3		5	6	5	4	4
16	9	M	Goitrous		2	—	3		4	4	3	2	2
17	12	M	Goitrous		3	2	3		4	4	3	3	3
18	4	F	Athyreotic		3	3	2		3	4	2	3	3
19	2	F	Dysgenesis		3	—	3	—	3	3	3	2	2
20	12	F	Goitrous		3	—	2	—	3	3	3	3	1
21	8	F	Goitrous	Mild	3	—	1	—	3	3	3	2	1
22	7	M	Goitrous		3	—	1	—	3	3	3	2	1
23		M	Athyreotic	Severe	3	—	2	—	3	3	3	2	1
			Range of 23 cases of hypothyroidism		1 5	0-3	1-4	Trace	2-5	2-6	2-5	2-4	1-6
			Range of 10 controls		0-2	0-2	0-1		2-3	1-3	1 3	2 3	1-3

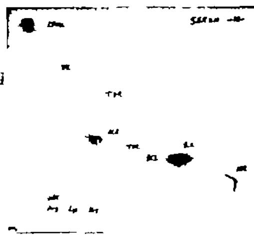



Fig. 1 Fig 2

Fig. 1 Bidimensional chromatogram of case 1
Fig. 2. Bidimensional chromatogram of normal control.

butanol/acetic acid/water was used as the second solvent [14]. Semiquantit tive assay of amino acids in the bidimensional method was carried out by visual comparison with chromatograms of standard mixtures of known content of amino-acids run and stai ned under identical conditions. A modified scoring system according t Chisolm [5] was used for semiquantitative assay of in dividual amino-acids using an approximate micro molar equivalent

Micro osmol / analytical aliquot	Arbitary numerical score
0 025	+1
0 05	+2
0.1	+3
0.2	+4
0.4	+5
0.8	+6

Urinary chromatograms were carried out on the same lines for the 12 cases using 20 microliters of fresh urine after being desalted electrolytically

Results

Table 1 summarizes the clinical fea tures of the 23 cases together with the scores for the individual serum amino-acids detected, and the range for 10 nor mal controls. An illustrative bidimen sional chromatogram is shown in Fig 1 (Case 1) compared with control (Fig. .).

It is evident from Table 1 that all cases showed an increase of serum amino-acids mainly affecting glutamic acid, alanine and valine. Leucine, arginine lysine and phenyl alanine showed a slight increase in most cases. The degree of amino-acidemia was not related to the age, duration or type of cretinism but was found to be related to the severity of the clinical picture (note Cases 20 21 and 22 with mild features of cretinism and minimal mino-acidemia)

The results for the 13 cases that were followed up after dequate thyroid therapy are shown in Table 2.

Urinary chromatograms for cases before and after treatment did not show any deviation from the normal pattern of controls.

Discussion

It is evident from our results that an increase in fasting serum amino-acids is

TABLE 2. *Scores for amino-acids before and after treatment*

No.	Age (Y)	Sex	Type of cretinism	Severity	Treat-ment	Leucine	P Alanine	Valine	Tyrosine	Alanine	Glutamic acid	Glycine	Aspartic	Arginine and lysine
						Scores of amino-acids in serum chromatograms								
1	4	M	Athyreotic		Before	5	3	3		5	3	5	4	6
					Under	3	—	1		3	2	3	2	3
2	5	F	Athyreotic		Before	3	3	2		5	4	5	4	3
					Under	1	—	1		3	2	3	2	1
3	4	F	Athyreotic		Before	3	—	2		4	4	3	3	2
					Under	1	—	1		3	2	2	1	1
4	5	M	Athyreotic		Before	4	3	3		5	4	5	4	1
					Under	2	—	2		3	3	3	3	1
5	3	M	Athyreotic		Before	3	3	3		5	6	4	4	4
					Under	3	3	3		3	3	3	3	3
6	10 m	F	Dysgenesis		Before	4	3	3		4	4	5	4	3
					Under	3	2	2		3	3	2	2	3
7	1	F	Goitrous	Severe	Before	4	3	3		5	4	4	4	3
					Under	1	—	1		3	3	3	2	1
8	9	M	Goitrous		Before	1	—	4	Trace	3	3	3	2	3
					Under	—	—	1		3	2	1	1	2
9	11	M	Goitrous		Before	3	—	3		4	3	5	4	3
					Under	2	—	2		3	3	3	3	2
10	1 /	F	Athyreotic		Before	2	—	2		4	3	4	3	2
					Under	1	—	2		3	1	3	2	1
11	2 /	F	Athyreotic		Before	2	—	3		3	4	4	3	4
					Under	2	—	3		3	3	2	3	2
12	2½/	F	Athyreotic		Before	3	—	3		4	3	3	3	3
					Under	2	—	2		3	2	2	1	1
13	4 /	F	Dysgenesis		Before	3	—	3		3	3	3	4	2
					Under	2	—	1		3	3	2	3	1
Range of 13 cases of cretinism					Before	1–5	0–3	1–4		2–5	2–5	2–5	3–4	1–6
					Under	1 3	0–3	1 2		2 3	1 3	1–3	1 3	1 3
Range of 10 controls						0–	0–2	0–1		2 3	1–3	1–3	2–3	1 3

present in all cases of congenital hypothyroidism studied. This amino-acidemia was generalized keeping the normal serum amino acid pattern. The increase mainly affected those amino acids that are dominant in normal plasma, i.e glutamic acid, glycine alanine and valine

Thyroid hormone exerts a dual effect on plasma amino-acid level. Through its protein anabolic action, promotion of active intracellular amino-acid transport, acceleration of the rate of incorporation of amino-acids by the liver [11], it tends to lower the blood amino-acid level. On the other hand through its catabolic action increasing protein degradation it tends to cause an elevation of blood amino-acid level. The net effect would depend on the balance between these various actions of thyroid hormone which is influenced by many factors including the previous thyroid status of the individual, dosage of thyroid hormone and the protein intake.

The increase in serum amino acids observed in congenital hypothyroidism is attributed to thyroid hormone deficiency with lack of its anabolic and other effects that tend to lower blood amino-acid level. As evident from the results milder cases of congenital hypothyroidism showed minimal changes in serum amino-acids. Impairment of the liver function might also be involved in this hyperamino-acidemia. The hypercarotinemia and neonatal hyperbilirubinemia occasionally observed in congenital hypothyroidism reflect the presence of functional hepatic impairment in that condition [1] Delayed B.S P excretion and impairment of some liver functions was recently observed in advanced cases of hypothyroidism [8].

Thyroid therapy resulted in lowering of serum amino-acids to within normal levels in 11 cases. In Cases 5 and 6 some degree of hyper-amino-acidemia was still present after adequate replacement therapy

There was no evidence from th present work that a specific amino-acid excess or deficiency existed in cases of hypothyroidism including goitrous cretinism, the hyper-amino-acidemia was always generalized. The absence of hyper-amino-aciduria indicates that thyroid hormone has no effect on the renal tubular reabsorptive capacity for aminoacids which acted efficiently even in the presence of the moderate hyper-amino-acidemia observed.

Summary and Conclusion

The amino-acid chromatographic patterns of the serum and urine in 23 cases of congenital hypothyroidism were studied semi-quantit tively An increase in serum amino-acids was present in all cases affecting mainly glutamic acid glycine alanine and valine. The increase was less evident in milder cases. Urinary amino-acids were within the normal range Adequate thyroid replacement therapy in 13 cases resulted in lowering of the hyper amino-acidemia to the normal range in 11 cases. The possible actions of thyroid hormone on the plasma amino-acid level are presented nd the results are discussed.

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From the Aid of Crippled Children Laboratory the Depatments of Obstetrics and Gynaecology Paediatrics and Educational Psychology University of Lund, Sweden

A Follow up Study of Premature Infants Treated with Low Oxygen Tension[1]

by G ROOTH, G ENGLESON and M. TÖRNBLOM

Introduction

In 1959 Sjöstedt & Rooth [4] and in 1960 Engleson, Rooth & Sjöstedt [2] reported the immediate results of some infants treated in incubators with 15% oxygen, i.e. in an atmosphere with an oxygen tension in between that of the intrauterine level and that of air

A total of 25 premature infants were treated in 15% oxygen for a period of 7–37 days, mean 25.5 days. Of these 11 died, 7 of these had a birthweight of less than 1300 g Taking regard to birthweight there were no differences in the neonatal mortality between the prematures treated in 15% oxygen and those prematures which during these years were treated by the Department of Paediatrics in Lund. No particular selection was used for choosing the cases for the incubators. As soon as one of the two incubators was available the next born premature infant was placed there. As there was no difference in the immediate mortality between the two groups of prematures it was of interest to see if there were any differences in the treated infants and the matched control at a follow-up study

[1] This study was supported by grant from the Swedish Medical Research Council.

Material and Methods

All the surviving premature infants treated in 15 oxygen in 1956 and 1957 were included in the follow-up study The infant were divided in groups according t birth weight: < 1000 g 1000–1299 g 1300–1599 g,

TABLE 1

	Premature infants				Term infants controls
	Low oxygen tension		Controls		
	13	n. 13	18	n. 19	
Mean total* score without intelligence test	10.2	12.7	11.6	7.3	
Terman Merill	95	99	91	110	

* Not included EEG and walking at the time of the follow-up

oxygen is intermediate between the premature and the term controls. The difference between the term controls and the 15% oxygen group is not significant but the difference between the normal term infants and the premature controls is significant ($t = 2.71$ $p < 0.01$ $n = 32$). Turning to the intelligence test the Terman Merill test as well as the WISC test were a few points better in the infants treated in 15% oxygen than in the premature controls, but this difference was not significant. Only the Terman Merill test could be used to compare the normal term infants with the present two groups. It was found that the term infants did significantly better ($t = 4.50$ $n = 51$ $p < 0.001$).

We tried to see if there were any particular variables, such as electroencephalogram, neurological findings or special groups within the intelligence test which could fit into a pattern which would distinguish the treated prematures from the normal prematures. No such pattern could be identified.

Discussion

There is no evidence to believe that the difference between the control group and the group treated in 15% oxygen is due to a biased selection. In spite of this there is some difficulty in the interpretation of the results as the treatment differed between the two groups in more than the oxygen environment. The controls were not treated in incubators and were at the Department of Paediatrics while the treated infants remained at the Department of Obstetrics and Gynaecology. Temperatures and other factors might also have differed.

Summary

Although no final conclusion may be drawn as to the value of treatment of premature infants in low oxygen tension the present results speak in favour of this treatment.

References

1. Engleson G, Rooth, G and Sjöstedt S: Treatment of premature infants with 15 oxygen. *Acta Paediat Scand Suppl 118* 1958.
2. Engleson G, Rooth, G and Törnblom, M: A follow-up study of dysmature infants. *Arch Dis Child, 38* 62, 1963.
3. Karlberg, P and Priwan A: Some physical measurements (weight, length, head circumference and chest circumference) in healthy Swedish children in the first two years of life. *Acta Paediat Scand Suppl 117* 128, 1959.
4. Sjöstedt S and Rooth, G: Low oxygen tension in the management of newborn infants. *Arch Dis Child, 32* 397 1957.
5. Wechsler, D: *Wechsler Intelligence Scale for Children.* New York, 1949.

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Methods and Materials

Patient material Erythropoietin assays were performed on plasma obtained from 39 children. Routine hematologic methods were employed in the diagnostic evaluation of their blood dyscrasias. In some instances, the anemia constituted the primary disease process and in others it was secondary to some other disorder

The plasma from five patients with plastic anemia was assayed. Three of these were idiopathic in origin insofar as could be ascertained by history In Case 3 there was history of exposure to several chemical agents which have been known to cause bone marrow depression Of particular interest is Case 2 in that a sibling of this patient died of hemorrhage secondary to bone marrow hypoplasia thus suggesting that these children had a congenital hypoplastic anemia without associated anomalies. Two patients showed a specific erythroid hypoplasia on bone marrow examination and had no associated peripheral blood leucopenia or thrombocytopenia. Two additional patients have been included with regenerative anemia. One of these had a maturation arrest with predominance of early and late erythroblast and the other an essentially normal bone marrow morphologically

Of the seven patients with acute leukemia, three were classified as lymphoblastic two monoblastic and two myeloblastic. The plasma of those patients was assayed during various stages of their leukemic process and six of them showed great predominance of blast cells in their bone marrow at the time of assay Case 15 had a hypoplastic bone marrow secondary to chemotherapy and Case 16 at the time of the assay had septicemia and meningitis due to Diplococcus pneumoniae

Nine patients with iron deficiency anemia formed a relatively homogeneous group with an age range of 8½ months to 18 months and nutritional inadequacy of iron was the major etiologic factor in the anemia. In addition, four of them (Cases 18, 20 22 and 23) had occult blood in the stool on at least one occasion. Case 22 is of special interest in that the child also had a congenital malformation of the right kidney which was interpreted radiographically as a "conglomerate renal collecting system.

Four patients with hemolytic anemias were also investigated, but this is a heterogenous group consisting of 1 case each of sickle cell anemia, erythroblastosis fetalis, idiopathic acquired hemolytic anemia, and nonspherocytic hemolytic anemia. The two cases of Letterer-Siwe disease can probably also be considered in the same category in that hypersplenism may be a major factor in the anemia of this disorder The patient with biliary cirrhosis had biliary atresia as the etiology for her cirrhosis and was approaching the terminal stage of her disease process. The hematologic picture was suggestive of increased red cell destruction and was probably in part due to secondary hypersplenism.

Of note among the three cases of acute glomerulonephritis is Case 35 who had renal failure necessitating repeated dialysis. Two patients had chronic glomerulonephritis. The patient with reticulum cell sarcoma had severely depressed bone marrow secondary to chemotherapy and also had bilateral renal tumor infiltration as demonstrated by excretory urography The child with Wilm tumor had had nephrectomy for one tumor and at the time of the assay had large tumor involving the other kidney manifest clinically as an abdominal mass which filled most of the abdominal cavity

Method Blood specimens from the above mentioned patients were anticoagulated with heparin or oxalate and the plasma was removed and frozen until the assay was performed For each assay ten rats of the Sprague-Dawley strain were used, five as controls and five as test animals. The initial weight of the rats was 100 to 200 g and food was withheld throughout the test period but water was given ad lib Twenty four and forty-eight hours after the onset of fasting, the rats were injected subcutaneously with 0.5 ml of the test plasma. The control rats received 0.5 ml of normal saline

TABLE 1 *The effect of plasma and urine from patients with blood dyscrasias on the Fe^{59} uptake in starved rats*

No.	Age	Sex	Diagnosis	Hgb. %	Hct. %	Retic. %	Bone marrow	Fe^{59} uptake % ± S.D.	Control
Patients with decreased red cell production									
1. K. H.	11 yr	M	Aplastic anemia	8.7	27	0.6	Hypoplastic	3.4 ± 1.5	3.0 ± 0.4
2. S. S.	11 yr	F	Aplastic anemia	2.0	6	3.9	Aplastic	22.5 ± 5.9*	3.2 ± 1.0
							Urine	10.3 ± 6.3	2.4 ± 3.7
3. S. L.	8 yr	M	Aplastic anemia	5.3	14	0.1	Aplastic	15.4 ± 6.4*	2.4 ± 0.7
4. M. H.	7 yr	M	Aplastic anemia	2.9	8	4.3	Aplastic	13.0 ± 4.8*	5.6 ± 2.1
							Urine	9.4 ± 3.4*	2.5 ± 2.0
5. M. M.	3 yr	M	Aplastic anemia	8.3	29	0.4	Aplastic	2.3 ± 1.[illegible]	[illegible].0 ± 1.0
6. T. K.	20 mo.	F	Hypoplastic anemia	5.6	18	0.4	Erythroid hypoplasia	21 ± 4.4*	5.4 ± 1.1
7. D. M.	4 mo.	M	Hypoplastic anemia	6.3	20	1.5	Erythroid hypoplasia	3.8 ± 3.6	2.7 ± [illegible].1
8. M. G.	3 yr	M	Aregenerative anemia	4.8	14	2.0	Erythroid hyperplasia	8.4 ± 4.1	7.1 ± 4.6
							Urine	1.6 ± 0.5	1.1 ± 0.1
9. K. L.	6 mo.	M	Aregenerative anemia	7.0	22	3.8	Normocellular	5.2 ± 1.6	5.3 ± 2.7
10. R. K.	11 yr	M	Monocytic leukemia	7.2	22	2.8	Leukemic	4.1 ± 1.9	4.0 ± 1.1
11. Y. C.	3 yr	F	Monocytic leukemia	5.5	18	1.4	Leukemic	3.7 ± 1.3	4.2 ± 4.7
12. D. K.	16 mo.	M	Myelocytic leukemia	5.4	16	1.0	Leukemic	10.4 ± 4.8*	3.5 ± 1.3
13. R. M.	1 mo.	F	Myelocytic leukemia	9.7	28	[illegible].0	Leukemic	8.7 ± 0.2*	4.5 ± 1.5
14. J. M.	6 yr	M	Lymphocytic leukemia	6.3	20	1.5	Leukemic	[illegible].4 ± 1.4	1.3 ± 0.5
15. M. K.	4 yr	M	Lymphocytic leukemia	6.3	18	0.6	Hypoplastic	1[illegible].9 ± 3.5*	2.9 ± 2.9
16. A. B.	3½ yr	M	Lymphocytic leukemia	3.9	12	1.3	Leukemic	21.4 ± 5.5*	11 ± 6.5
17. R. M.	6½ mo.	M	Iron deficiency	2.1	10	4.1	Normoblastic hyperplasia	7.0 ± 2.1	5.4 ± 0.7
18. D. F.	11 mo.	F	Iron deficiency	2.9	13	1.5	Normocellular	7.7 ± 2.3*	4.4 ± 2.9
19. D. S.	18 mo.	M	Iron deficiency	5.4	24	4.1	Normoblastic hyperplasia	6.5 ± 1.5	5.0 ± 3.3
20. J. H.	16 mo.	M	Iron deficiency	4.3	19	4.5	Normocellular	10.6 ± 4.5*	3.5 ± 0.6
21. D. W.	16 mo.	F	Iron deficiency	5.3	19	4.1	Normoblastic hyperplasia	3.7 ± 1.0*	1.7 ± 0.7
22. G. K.	14 mo.	M	Iron deficiency	3.0	12	3.1	Not obtained	19.7 ± 7.8*	6.7 ± 1.4
23. D. B.	12 mo.	M	Iron deficiency	4.4	18	1.0	Normocellular	[illegible].2 ± 1.1	1.1 ± 0.4
24. M. B.	13 mo.	M	Iron deficiency	4.8	22	2.9	Not obtained	6.9 ± 3.6*	1.9 ± 0.5
25. B. B.	10 mo.	M	Iron deficiency	4.0	20	6.7	Not obtained	11.3 ± 3.5	8.8 ± 4.1
							Urine	7.9 ± 1.5*	2.2 ± 0.9
Patients with increased red cell destruction									
26. R. V.	13 yr	F	Idiopathic acquired hemolytic	6.7	18	38.0	Erythroid hyperplasia	7.7 ± 5.0	3.6 ± 0.6
27. M. Q.	2 days	F	Nonspherocytic hemolytic	7.5	20	4.8	Erythroid hyperplasia	1.3 ± 0.6	1.4 ± 0.[illegible]
28. B. T.	10 hr	F	Erythroblastosis fetalis	10.7	30	3.0	Not obtained	4.0 ± 1.6	4.6 ± 3.2
29. A. K.	5 yr	F	Sickle cell anemia	7.5	24	15.0	Erythroid hyperplasia	3.8 ± 1.5	2.3 ± 0.5
30. M. D.	13 mo.	F	Letterer-Siwe disease	5.3	17	4.4	Hyperplastic	1.5 ± 0.7	1.1 ± 0.4
31. J. K.	7 mo.	M	Letterer-Siwe disease	6.9	23	[illegible].9	Hyperplastic	2.9 ± 1.4	2.0 ± 0.7
32. B. D.	23 mo.	F	Biliary cirrhosis	7.7	21	4.4	Erythroid hyperplasia	1.4 ± 0.6	2.1 ± 1.0
Patients with renal disease									
33. J. O.	6 yr	M	Acute glomerulonephritis	8.5	30	1.5	Not obtained	0.4 ± 0.2	1.2 ± 0.1
34. S. O.	8 yr	M	Acute glomerulonephritis	8.7	29	1.0	Not obtained	1.2 ± 0.3	1.2 ± 0.1
35. M. J. M.	9 yr	F	Acute glomerulonephritis	5.9	20	0.6	Not obtained	4.6 ± 1.7	3.7 ± 1.3
36. G. H.	15 yr	M	Chronic glomerulonephritis	6.8	20	0.4	Not obtained	4.4 ± 1.5	3.8 ± 2.1
37. S. M.	13 yr	F	Chronic glomerulonephritis	6.8	22	0	Not obtained	3.6 ± 1.8	3.5 ± [illegible].1
38. B. H.	13 yr	M	Reticulum cell sarcoma	6.3	16	0	Hypoplastic	2.6 ± 0.6	2.9 ± 1.0
39. J. P.	18 mo.	F	Wilm tumor—bilateral	7.0	20	1.8	Not obtained	6.7 ± 1.7*	3.[illegible] ± 0.5
			Control—703 assays /saline injections					3.8 ± 2.13	

* Fe^{59} uptake is significantly different from control, $P < .05$.

Discussion

Erythropoietin plasma levels are dependent on the degree of hypoxia, i.e. the severity of the anemia and the erythroid activity of the bone marrow [1 30] The effect of the degree of hypoxia has been demonstrated in experimental animals both by varying barometric pressure and by varying the severity of blood loss anemia [30]. The greater the hypoxia, the more erythropoietin activity is demonstrable.

The effect of the erythroid activity of the bone marrow was studied by exposing irradiated and non-irradiated rats to a simulated altitude of 23 000 feet Higher erythropoietin levels were demonstrated in the irradiated group. In addition, the rate of disappearance of erythropoietin was approximately doubled in the irradiated group [29 30]. Thus when erythropoiesis is active the erythropoietin is utilized in the process but bone marrow depression permits this substance to accumulate in the blood.

Erythropoietin in part initiates the differentiation of stem cells to erythrocyte precursors but evidence also suggests that it acts on later stages of erythrocyt maturation [16 31] Stohlman further postulates that it initiates hemoglobin synthesis until a critical cytoplasmic concentration is reached At that point, hemoglobin synthesis stops and a negative feedback mechanism prevents further cell division [31].

Having thus briefly outlined the current concepts of the mechanism of action of erythropoietin, the results of the assays performed in this study can be looked at more logically The anemia secondary to plastic or hypoplastic bone marrow results in elevated erythropoietin blood levels. The hypoxia of the anemia stimulates its production and the depletion of the stem cell compartment along with other erythrocyte precursors decreases utilization. The refractory anemias fall into a different category since red cell precursors are present in the bone marrow and a maturation arrest (ineffective erythropoiesis) exists. The erythropoietin levels in the patients reported here did not differ significantly from their controls. In one instance the hemoglobin was 7 0 g/100 ml which may not be sufficient stimulus to produce demonstrable erythropoietin. The hemoglobin of the other patient was 4.8 g/100 ml which is comparable to the anemia of the patients with depressed bone marrows and elevated values. Since the stem cell compartment apparently is not depressed, perhaps the erythropoietin is in part utilized by the marrow and relatively greater degrees of anoxia are required before elevated blood levels are demonstrable.

The anemia of acute leukemia is in part similar to that of aplastic anemia in that there is a paucity of erythroid precursors in the marrow In addition, hemorrhage and hemolysis may contribute to the anemia. The explanation for normal values in two of the patients is not readily ppa rent.

In iron deficiency anemia, the hemoglobin must be below 4–5 g/100 ml before erythropoietin is demonstrable [30 34]. The data presented here are in keeping with these findings. Stohlman postulates that this finding is a reflection of a relatively active bone marrow [31].

Similar results have been found by other investigators assaying plasma from

Erythropoietic stimulating activity has also been found in the fluid of renal cysts and tumor extracts of hypernephromas [14]. Experimentally polycythemia can be produced by unilateral partial ligation of the renal vein [*0]. It would thus seem that severe renal insufficiency depresses red cell production and that the kidney in certain other types of renal disease is capable of increasing red cell production.

Both animal and human data thus far obtained has failed to demonstrate erythropoietin except in the presence of severe anemia [18, 33 35] and in some instances of polycythemia or erythrocytosis. The assay techniques may not be accurate and precise enough to detect small amounts of erythropoietin. Another explanation could be the rapid utilization by the bone marrow so that no excess accumulates in the blood normally A third possibility is that erythropoietin is only called into play in the presence of severe anemia and some other mechanism accounts for the fine regulation of red cell production. It is likely that nutritional [., 27] and endocrine [31] factors also enter into the regulation of erythropoiesis.

Summary

Erythropoietin assays were carried out on the plasma of 39 children with various blood dyscrasias. Increased erythropoietin activity was found primarily in those patients with depressed bone marrow function and severe anemia No direct relationship could be found between the severity of the anemia and the erythropoietin level. No erythropoietin was found in the plasma of patients with hemolytic anemia or renal disease with the exception of one patient with bilateral Wilm tumor

References

1 Brecher, G. and Stohlman F Jr.: Humoral factors in erythropoiesis, In: Tocantins, L. M., Ed., *Progress I Hematology* Vol. *II* New York, Grune and Stratton, 1959, p. 110.
2. Erslev A. J The role of erythropoietin in the control of red cell production. *Medicine*, 43. 661 1964.
3. Fried W Plzak, L. F and Jacobson L. O Studies on erythropoiesis. III. Factors controlling erythropoietin production. *Proc Soc Exp Biol Med 94* 237 1957
4 Gallagher, N L, McCarthy J M., Hart K. T and Lange, R. D Evaluation of plasma erythropoietic-stimulating factors in anemic uremic patients. *Blood 14* 662, 1959
5. Gallagher, N L, McCarthy J M. and Lange, R. D Observations on erythropoietic-stimulating factor (ESF) in the plasma of uremic and non-uremic anemic patients. *Ann Intern Med, 52* 1201 1960
6. Goldwasser, E., Fried W and Jacobson L. O Studies on erythropoiesis. VIII. The effect of nephrectomy on response to hypoxic anoxia. *J Lab Clin Med, 52* 375, 1958.
7 Gurney C. W and Pan C Studies on erythropoiesis. XIII A comparison of methods of bioassay of erythropoietin in human plasma. *J Lab Clin Med 55* 67 1960.
8. Gurney C W Goldwasser, E. and Pan C Studies on erythropoiesis. VI. Erythropoietin in human plasma. *J Lab Clin Med, 50* 534, 1957
9 Hammond, G D., Ishikawa, A. and Keighley G Relationship between erythropoietin and severity of anemia in hypoplastic and hemolytic states, In: Jacobson, L. O and Doyle M., Eds., *Erythropoiesis*. New York, Grune and Stratton, 1962, p. 351
10 Jacobsen E. M., Davis, A. and Alpen E L.: Relative effectiveness of phenylhydrazine treatment and hemorrhage in the production of an erythropoietic factor *Blood 11* 937 1956.
11 Jacobson L. O Goldwasser, E., Fried W and Plzak, L.: Role of kidney in the control of erythropoiesis. *Nature (London)*, *179* 633, 1957
12. Jacobson, L. O Goldwasser, E., Gurney C. W Fried, W and Plzak, L. F Studies of erythropoietin: The hormone regulating red cell production. *Ann NY Acad Sci, 77* 551 1959

crystals and increased excretion of cystine lysine arginine and ornithine were detected. Bone marrow examination was negati Many stones in the pelvis, ureters and even urethra were found. Penicillamine was administered with excellent results

Case 3

A. D woman, 40 years old t the death, was hospitalized for the first time t the age of 4 years because of bilateral renal stones and pyelonephritis. Until the ge of 37 she suffered from repeated urinary tract infections and renal colics. Then, hypertension and proteinuria ppeared and on I. pyelography bilateral stones were found One of the oided calculi was found composed of pure cystine At the age of 39 years renal failure developed and blood pressure rose to 230/135. Chromatography of the urine was normal. Left ureterolithotomy was performed. One year later the patient expired during an ttack of acute pyelonephritis due to E.coli and renal failure. At utopsy contracted pyelonephritic kidneys with many cystine crystals in the tubuli were found. The small arterioles had prominent thickening on the media.

Comments to the case. In this woman hypertension appeared after suffering of 33 years of renal stones and urinary tract infections. Two years later renal failure developed. Therefore normal chromatographic pattern may be explained on the base of lowered glomerular filtration. Changes in the renal arterioles were well correlated with the hypertension which was caused by chronic pyelonephritis probably

Case 4

B A., a 39 year-old woman was operated at the age of 7 years because of right renal calculi. After this she suffered from repeated renal colics and urinary tract infections. At the age of 15 years I.v py lograph showed bilateral nephrolithiasis nd two years later left ureterolithotomy was performed. Urea was then 58 mg% and U.C.T 24% At the age of 21 22 man stones spontaneously oided were found composed of pure cystine Until the age of 31 years, the general condition was satisfactory xcept for repeated E.coli urinary tract infections. Then, renal colics recurred. Urea was 68 mg uric acid 7.5 mg%, calcium and phosphorus normal Chromatography f the urine revealed ery increased amounts of cystine and lysine and increased quantities of arginine phenylalanine, glycine, and alanine The le el f blood amino acids w 3 6 mg% No disturbances of carbohydrat metabolism were detected. From the age of 31 t 39 small stones were spontaneously oided twice yearly in the verage Urea w s normal. I v pyelography did not show any findings. Creatinine clearance was impaired

Comments to the case. This woman suffered from the ge of 7 years from repeated formation of cystine stones and urinary tract infections. After 31 years of follow up the general condition is good and renal functions are only slightly disturbed.

Case 5

A. B 17 year-old girl suffered t the age of 10 years from "nephritis with blood in the urine At the age of 12, she was hospitalized because of renal colic. Blood pressure was 120/80 Urea 130 mg In the urine protein and many cystine crystals were detected. Chromatography was typical for cystinuria. Urinary culture revealed E. coli. I v pyelography showed bilateral hydronephrosis with staghorn stones. Bilateral pyelolithotomy was performed. The urea fell to normal. Many liquids and lkali citrate were administered From the age of 12 years to 17 years occasional renal colic appeared. Familial in vestigation showed that the mother and 6 siblings suffered from cystinuria. In the brother who i 19 years old, vesical stone was detected and the second brother 8 years old, suffers from renal colic.

predominate [38]. Cystine stones are reported to be much more frequent in the males—70-80 [43]. The age of cystinuric patients when the disease is discovered varies from the infancy to the ninth decade of life [5 12].

Genetics Harris et al. divided the inheritance of cystinuria into recessive and incomplete recessive types. In the first type only homozygotic persons are clinically involved, xcrete large amounts of amino acids and produce stones In the second type in addition to homozygotics similar to the first type also heterozygotics excrete increased amounts of amino acids but in a much lower proportion. They do not form any stones, practically [3.].

Metabolism The level of plasma cystine in cystinuri patients is normal or very slightly lowered [26, 31 36]. Low values were reported only once [19]. On the other hand, the level of plasma cysteine was found definitely low 0.00-0.09 mg as compared with 0 14-0.54 mg* in normal persons, and the amount of urinary cysteine was found clearly reduced [9].

Five amino acids are overexcreted in the urine—cystine lysine arginine ornithine and mixed disulphide of cysteine and homocysteine The amount of the last amino acid is 5-15 of that of excreted cystine and reaches the values of 15-224 mg/24 hrs [30 31].

It seems that kidneys excrete cystine in normal amounts and its increased quantities in the urine arise from plasma cysteine Renal arterio-venous difference was found normal for cystine whereas cysteine was overexcreted [31] Cystine transport in the kidney tissue lices is normal [*] but the transport of lysine and arginine was found defective [3.].

Experimental data showed that feeding of cystine to cystinuric patients did not influence the amounts of urinary cystine whereas feeding of cysteine increased it clearly [41 51]. Feeding of cysteine also produced an elevation of the plasma level of cystine more in cystinuric patient than in controls [19]

It has been shown recently that the intestinal transport of dibasic amino acids is impaired in cystinuria. They re absorbed incompletely and may be detected in the feces [2, 5.]. Defective intracellular accumulation of these amino acids in the epithelial duodenal cells was found [4].

It may be summarized t present that defective transport of dibasic amino acids probably enzymatic exists in both renal and intestinal systems. Excessive increase of plasma cystine after ingestion of cysteine suggests the existence of more general metabolic disturbances.

Although large amounts of dibasic amino acids are excreted in the urine and probably incompletely absorbed by the intestinal tract no signs of malnutrition or deficiency were reported in cystinuric patients [5]. Only Collis et al. found that the height of 44 homozygotic persons was slightly lower than that of the general population [13].

Cystine contents in hair and fingernails of cystinuric children was found normal [40]. These reports appeared in the period when no clear distinction was made between cystinosis and cystinuria, however

Sweat and saliva of cystinuri patient contain normal amounts of amino acids [7] and leucocytes are able to incorporate labeled cystine and lysine at a normal rate [3].

In experimental animals cystine defi-

autopsy contracted hydronephrotic kidneys were found. Glomeruli were destroyed, tubuli atrophied and severe changes in the arterioles with media hypertrophy fibrous endarteritis and fibrinoid necrosis were detected. Accord ing t the authors it was a combination of cystine lithiasis and nephritis. N cystine crystals were found in the internal organs and the tissues [49]. It is not clear if the child suffered from nephritis or rather from chronic pyelonephritis.

Cases of arterial hypertension and changes in the renal arterioles in children with uni or bilateral chronic pyelonephritis ha e been reported [6, 47].

Frank et al. (Case 1) described a child which died at the age of 12 years of renal and heart failure. From the age of 9 years he suffered from hematuria and t the age of 11 many cystine stones were found. Urinary tract infections, renal failure and hypertension up to 220/140 developed At autopsy right hypoplastic kidneys with hydronephrosis and hydroureter were detected. Microscopically all the signs of chronic pyelonephritis were found in both kidneys. No cystine crystals were seen in the internal organs or tissues. Atheromatous changes were found in the coronary arteries and bdominal aorta [29]. It is not clear if the hypoplastic kidney was of congenital origin or connected with long standing back pressure due to stones and chronic pyelonephritis. Powers & Murray reported a case of a 6-year old boy with severe hypertension, chronic pyelonephritis and congenitally hypoplastic kidney [47].

In our series blood pressure of 135/75 was found in an infant of 7 months who suffered from bilateral cystine lithiasis. At autopsy thickening in the media of kidney arterioles was observed.

Connection of Cystinuria With Other Disorders

Cystinuria with severe steatorrhea [25] and with celiac disease [33] were reported in children. Familial chronic pancreatitis with cystinuria was also described [3?].

Increased excretion of cystine was reported in cases of mental retardation with atypical osteogenesis imperfecta [4], and mental deficiency with epilepsy [54]. A case of an epileptic child with cystinuria and cystine stones was also reported [37]

Cystinuria with hemophilia and cystine lithiasis with retinitis pigmentosa were described [16].

Congenital malformations such as con genital goiter spina bifida, double ureter agenesis of the kidney phimosis and stra bismus were described in cystinuri pa tients [10, 28, 48].

Cystinuria with more general amino aciduria was reported Glucine, serine, tyrosine and other mino acids were overexcreted in two cystinuric children [?1]. In our material there were two cases with overexcretion of additional amino acids, such as glycine leucine phenyla lanine alanine histidine taurine tyrosine and serine

Therapy

Increase of the urinary output to ml/ min, alkalisation of the urine [22 51] and administration of d(−)penicillamine [14 15] are the chief therapeutic methods. All these procedures are introduced to increase the amount of cystine in soluble state and to prevent stone formation. Therefore they should be pplied for very long time usually years. D(−)peni cillamine produces significant decrease of cystine excretion and does not influence the overexcretion of other amino acids. Very good results obtained by this drug in children suffering from cystine lithiasis were reported [15 44]. However side

effects such as development of nephrotic syndrome pyridoxine deficiency and blood disorders and even fatal cases due to agranulocytosis were reported [1 15 53].

Summary

Among 56 cases of cystinuria in fifteen (27) the disease was discovered at the preadolescent age Eight out of 21 cases of cystine urolithiasis began to suffer in their childhood which gave the rate of 38%—much higher than it is usually encountered in the literature. Renal colic, hematuria urinary retention and urinary tract infections may appear very early and lead subsequently to renal failure The stones are almost always bilateral and multiple Repeated surgical procedures are frequent.

Cumulative data on 31 children with cystine stones and prolonged follow-up in 24 of them showed that the disease is serious and sometimes fatal Four patients with cystine lithiasis died at young ages, which gives a mortality rate of 13 %.

An infant with bilateral cystine lithiasis and hypertension expired at the age of 7 months, is a unique and youngest case reported in the literature

It seems that cystinuria and cystine lithiasis are more frequent in infancy and childhood than it was believed before and that it is a serious and sometimes fatal disease Consequently in each discovered case a regular and prolonged therapy should be undertaken.

References

1 ADAMS, D A GOLDMAN R., MAXWELL, M H and LATTA H Nephrotic syndrome associated with penicillamine therapy of Wilson's disease *Amer J Med* 36 330, 1964

2. ASATOOR, A. M LACEY B. W LONDON D R and MILNE, M D Amino acid metabolism in cystinuria. *Clin Sci*, 23 285, 1962.

3 BECKER, F F and GREEN H Incorporation of cystine and lysine by normal and cystinuric leucocytes. *Proc Soc Exp Biol Med* 99 694, 1958

4 BERRY H K: Cystinuria in mentally retarded siblings with atypical osteogenesis imperfecta. *Amer J Dis Child* 97 196, 1959

5. BOSTRÖM, H. Cystinuria in Sweden. III The prognosis of homozygous cystinuria. *Acta Chir Scand*, 116 257 1959

6. BOSTRÖM, H. and HAMBRAEUS, L.: Cystinuria in Sweden, VII Clinical, histo-pathological and medico-social aspects of the disease *Acta Med Scand Suppl 411* 1964.

7 BOSTRÖM, H and TOTTIE, K.: Cystinuria in Sweden, II The incidence of homozygous cystinuria in Swedish schoolchildren. *Acta Paediat Scand*, 48 345, 1959

8. BOYCE, A. E.. Pyelonephritis in children and adults with hypertension. *J Urol* 42 960 1939.

9 BRIGHAM, M. P STEIN H. and MOORE, S.: Concentration of cysteine and cystine in human blood plasma *J Clin Invest* 39 1633, 1960.

10. CHARLEUX, G and MARCEL, J Lithiase cystinique de la vessie: cystinurie sans cystinose chez un enfant de 4 ans. *Pédiatrie* 8 946, 1953.

11 CHRISTIAENS, L., BISERTE, G FONTAINE, G and DESCAMPS, C.: La cystinurie (étude clinique biochemique et génétique). A propos de 3 familles. *A Pediat* 36 5, 1960.

12 CHRISTIAENS, L., WEMEAU L. and FONTAINE, G Lithiase rénale par *cystinurie* familiale *Arch Franc Pédiat* 16 704 1959.

13. COLLINS, J E., LEVI, A. J and MILNE, M. D.: Stature and nutrition in cystinuria and Hartnup disease *Brit Med J 1* 590, 1963.

14 CRAWHALL, J C., SCOWEN E. F and WATTS, R. W E.: Effect of penicillamine on cystinuria *Brit Med J I* 588, 1963.

15. — Further observations on use of D-penicillamine in cystinuria. *Brit Med J I* 1411 1964.

16. DENT C E. and HARRIS, H.: The genetics of cystinuria *Ann Eugenics 16* 60, 1951

17 DENT C. E. and ROSE, G A.: Amino acid metabolism in cystinuria. *Quart J Med, 20* 205 1951

18. DENT C. E. and SENIOR, B Studies on the treatment of cystinuria. *Brit J Urol, 27* 317 1955.

19. DENT, C. E., SENIOR, B and WALSHE, J. M.: The pathogenesis of cystinuria. II. Polarographic studies of metabolism of sulphur containing amino-acids. *J Clin Invest, 33* 1216, 1954

20. DEILL, V A.: Hepatotoxic agents: mechanism of action and dietary interrelationship. *Pharmacol Rev 4* I, 105...

21 EBERLEIN W P Aminoaciduria in childhood: cystinuria and cystinosis. *Amer J Med Sci, 225* 677 1953.

— EISENBERG, H., CONNOR, T B. and HOWARD J E.: A useful agent for oral alkali therapy *J Clin Endoc Metab, 15* 503, 1955.

23. FISCHL, J SABOV I and SEGAL, S.: A rapid spot test for the determination of cystinuria and aminoaciduria. *Clin Chem 7* 674, 1961

4. FISCHL, J and SEGAL, S.:) "Small-volume" electrophoretic separation of amino acids. b) Electro-chromatography *Clin Chim Acta, 8* 390, 479 1963.

25. FLEMING, W J AVERY G B., MORGAN R. L and GONE, JR., T E.: Gastrointestinal malabsorption associated with cystinuria. Report of case in negro. *Pediatrics, 32* 358, 1963.

26. FOWLER, D L., HARRIS, H. and WARREN F L.: Plasma cystine levels in cystinuria. *Lancet I* 544, 1952.

27 FOX, M., THIER, S ROSENBERG, L., KISER, W and SEGAL, S.: Evidence against single renal transport defect in cystinuria. *New Engl J Med 270* 556, 1964

28. FRANK, M., ATSMON A., YELLIN, G LA TKOVIC, J and DE VRIES, A.: Nephrolithiasis in Israel. A study of its incidence etiology and treatment. *Proc Staff Meeting Beilinson Hosp*, No. 10 1961.

29 FRANK, M DE VRIES, A., ATSMON, A., LAZEBNIK, J and KOCHWA, S Cystine lithiasis. *Harefuah, 55* 84, 1960.

30 FRIMPTER, G W The disulfide of l-cysteine and l homocysteine in urine of patients with cystinuria. *J Biol Chem, 236* 51 PC, 1961

31 — Cystinuria: metabolism of the disulfide of cysteine and homocysteine *J Clin Invest, 42* 1956, 1963.

32. GROSS, J B., ULRICH, J A. and MAHER, F T Aminoaciduria in family with hereditary pancreatitis. *Gastroenterol, 43* 784, 1962.

33. HAMBRAEUS, L., and DE HEVESY G Cystinuria in Sweden. VIII A case of coeliac disease associated with cystine lysinuria. *Acta Paediat Scand, 53* 213, 1964

34. HARRIS, H., MITTWOCH, U ROBSON E. B and WARREN F L The pattern of amino-acid excretion in cystinuria *Ann Human Genet, 19* 196, 1955.

35. — Phenotypes and genotypes in cystinuria. *Ann Human Genet, 20* 57 1955

36. HARRIS, H. and WARREN F L.: Quantitative studies on the urinary cystine in patients with cystine stone formation and in their relatives. *Ann Eugenics 17* 1 5 1953.

37 HERMAN L. and LEE, W E.: Cystine nephrolithiasis. Report of two cases *Am Surg 101* 746, 1935.

38 KNOX, W E.: Sir Archibald Garrod's Inborn Errors of Metabolism I. Cystinuria. *Amer J Human Genet 10* 3, 1958.

39. LEWIS, H. B The occurrence of cystinuria in healthy young men and women. *Ann Int Med 6* 183, 1932.

40. LEWIS, H. B. and FRAYSER, L. The metabolism of sulfur XXII. The cystine content of the hair and nails of cystinurics *J Biol Chem, 110* 23, 1935.

41 LOUGH, S A., PERLZWEIG W L HEINZEN H. J and CARTER, L. W.: Cystinuria The metabolism of cystine cysteine methionine homocystine and s-carboxymethylcysteine *J Biol Chem, 139* 487 1941

42. MCCARTHY C E., BORLAND JR. J L., LYNCH, JR. H. J., OWEN E. E. and TYOR, M. P Defective uptake of basic amino acids and L-cystine by intestinal mucosa of patients with cystinuria. *J Clin Invest, 43* 1518, 1964.

43. MORRISON H. R.: A roentgenological study of cystine urinary calculi. *Amer J Roentgen, 44* 537 1940.

44. MUNDEL, G FISCHL, J and YARRANTO, D Therapeutic trial in cystinuria with d(–)penicillamine (To be published.)

45 NOSSEN, S., ESSEX A. and BERNSTEIN A.: Cystinuria during pregnancy *Obst Gynec, 18* 187 1961

46. PERRY J W MOORE, A. E. and MACDONALD W B.: The diagnosis of aminoaciduria in childhood: cystine storage disease and cystine-lysinuria. *Med J Austral 2* 612, 1954.

47 POWERS, J H and MURRA M. F J venile hypertension associated with unilateral lesions of the upper urinary tract. *JAMA 118* 600, 1942.

48 PRCHAZKA, W Cystinuria—Report on 50 cases. *Urol Int* (in press).

49 RUSSELL, D. S and BARRIE, H.: Storage of cystine in the reticulo-endothelial system and its association with chronic nephritis and renal rickets. *Lancet, II* 899 1936

50 SCHWARZ, K.: Factor 3, selenium and vitamin E. *Nutrit Rev 18* 193, 1960.

51 SMITH, D R., KOLB, F O and HARPER, H A.: The management of cystinuria and cystine stone disease *J Urol, 81* 61 1959

52 THIER, S., FOX, M., SEGAL, S and ROSENBERG, L. E.: Cystinuria: in vitro demonstration of an intestinal transport defect *Science, 143* 482, 1964.

53 V… n, L., Lotez, P. and R… re, J. Étude chromatographique de l'amino-acidurie dans la cystinurie familiale. Arch. Franç. Pédiat. 10: 01, 19 3.
…4 V… ourt, J. K. and Hyr… I. … Lancet Fen 6: 112, 196…
55 W… r, J. M. Penicillamine. Practitioner 191: 7, 1963.
…6 Westall, R. G. The free amino acids of body fluids and some hereditary disorders of amino acid metabolism. Amino Acid Pools, edited by J. T. Holden. Elsevier Publ. Co., 196…
…7 Yeh, H. L., Fr… s, W., Dex…, M. S., L… ra, F., Hro… r, R. and Grön…, P. The urinary excretion of amino acids by cystinuric subject. Amer. J. Med. Sci. 214: 507, 1947.

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CASE REPORT

Total Tracheopulmonary Agenesis

Associated with Asplenia Agenesis of Umbilical Artery and Other Anomalies

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Major anomalies of the respiratory system are rare Olcott & Dooley [12], reporting a case of unilateral pulmonary agenesis, recorded that in 10,000 necropsies at the New York Hospital, many performed on newborn infants, there had been no previous instance of absence of a lung. They further cited Ellis s [5] report that Théremin had found only 2 cases in the records of 30 000 autopsies performed at a foundling hospital. Such figures indicate the infrequency of the anomaly Schneider [15] proposed a classification which has been followed by many subsequent authors.

1 AGENESIS—absence of lung bronchus and vascular structures on one side
2 APLASIA—trachea possesses a rudimentary bronchus but neither pulmonary alveolar tissue nor vascular structures are present.
3. HYPOPLASIA GRAVISSIMA—poor development of the main bronchus which fuses into a small mass of pulmonary tissue the bronchi in this mass are frequently dilated and the alveoli usually teleetatic

Oyamada, Gasul & Hollinger [13], in an extensive review of the literature on pulmonary agenesis accepted 74 cases as belonging to Schneider s classes 1 and 2. They listed a further 39 cases of congenital hypoplasia, and 21 cases of either agenesis or hypoplasia in which description was incomplete More recent reports include those of Marsoni [11] and Jones & Howell [10]. The total number of accepted cases of agenesis aplasia and severe hypoplasia is of the order of 100 at present Unilateral agenesis is not incompatible with life several patients have been first diagnosed in the fourth and fifth decades and at least one has lived to the age of 7 [8].

Thus the unilateral condition though uncommon, is not rare Congenital absence of both lungs is very rare, only 4 cases being recorded. Schmit [14], Allen & Affelbach [1] and Claireaux & Ferreira [4] each recorded a case of bilateral agenesis. Tuynman & Gardner [*0] reported an example of bilateral plasia. Bilateral bronchopulmonary agenesis associated with absence of the trachea and respiratory portion of the larynx (total tracheopul-

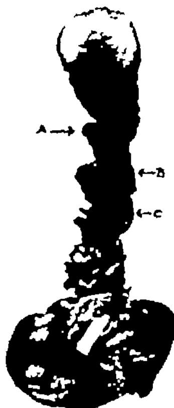


Fig 1 Posterior view showing hypoplastic la... (A) right lobe of the root (B) and cervical accessory h... III (C) Mark... sternal and superior ...

monary agenes) does not appear to have been recorded This prompted the following case report

Case Report

The mother was aged ... She was Group O Rhesus positive and the Wasserman reaction was negative Her two previous pregnancies resulted in term deliveries of normal male children weighing 3750 and 3000 g The father of the third child differed from that of the first two The last menstrual period was September 4th, 1963 Apart from some hyperemesis she was in good health during the first part of pregnancy Later detailed enquiry failed to reveal any evidence of viral infection or ingestion of drugs during the early weeks. Acute hydramnios ensued during the thirtieth week but an X-ray for foetal abnormality showed only a hemivert... at the ... lumbar junction. Repeated paracenteses were required ... times, the last ... week ... of ... from 1 14 to 2.2 l ... being removed on each occasion. Labour began spontaneously in the thirty-eighth week and 4 hours later resulted in ... normal delivery of a ... female infant weighing ... g The ... appeared normal but ... respiratory activity ... not ... through the chest ... moved several times. The heart beat was regular and the rate normal Attempt to intubate failed and on laryngoscopy only ... dimple was seen in the retroepiglottic tissues. All resuscitation measures failed and the heart finally stopped ... minutes after delivery

Necropsy findings

The body was that of a small newborn female infant weighing 2050 g and measuring 44 cm in crown-heel length. External development appeared normal The skull was normal and the falx and tentorium intact The brain was normal externally and on section. The spinal cord was normal The skeleton was normal apart from hemivertebra at the dorsolumbar junction.

On opening the abdomen only one umbilical artery was present The diaphragm was elevated, reaching the lower border of the third rib on the right and the upper border of the fourth rib on the left The liver was considerably enlarged, weighing 190 g inferiorly it protruded two finger breadths below the costal margin, laterally a substantial portion was present to the left of the mid-line though not reaching the lateral abdominal wall. The stomach was rudimentary measuring 2.5 cm between oesophageal and pyloric orifices and the lumen was very small. The spleen was absent The remainder of the intestinal tract pancreas biliary system, adrenals, kidneys, ureters bladder and genital organs were all normal. The anus was patent When the thorax was opened the heart and pericardial sac appeared to fill the

Comparison of reported cases of bilateral agenesis

	Schmit [14]	Allen & Affelbach [1]	Tuynman & Gardner [20]	Claireaux & Ferreira [4]	Present case
Gestation	9 months	Term	Term	Term	38 weeks
Sex	Not stated	Female	Male	Male	Female
Length	44.5 cm	47 cm	52 cm	48 cm	44 cm
Weight	Not stated	2330 g	3090 g	2500 g	2050 g
Larynx	Well developed	Not stated	Well formed	Normal	Upper (pharyngeal) portion only
Trachea	10 rings not separated from oesophagus	Normal, ended at root of heart	Well formed; 14 rings	3 poorly formed rings ended blindly	Absent
Bronchial buds	Absent	Primitive buds	Rim to left & longer right bronchial bud	Absent	Absent
Oesophagus	Fused with trachea	Normal	Normal	Normal	Normal
Heart	4 chambers	4 chambers	4 chambers	4 chambers	4 chambers
Superior vena cava	Not described	Not described	Normal	Right & left both present	Right & left both present
Inferior vena cava	Not described	Not described	Entered left atrium	Normal	Normal
Right atrium	Not described	Not described	Slightly dilated	Normal	Enlarged; persistent left superior vena cava & left azygos vein entered it; drained by common atrio-ventricular bicuspid valve
Foramen ovale	Patent	Patent—6 mm	Anatomically closed	Defect in atrial septum	Defect in atrial septum in anomalous site—7 mm
Right ventricle	Not described	Not described	Slightly dilated	Not described	Truncus arteriosus arose from it
Pulmonary artery	Entered aorta as ductus	Entered aorta at usual site of ductus	Entered aorta at usual site of ductus	Entered aorta distal to coarctation	Absent
Pulmonary veins	Absent	Absent	Absent	Absent	Absent
Left atrium	Received no vessels	Received no vessels	Received inferior vena cava	Received persistent left superior vena cava	Received no vessels; possessed mitral valve; opened into right atrium just above common atrioventricular [illegible]
Left ventricle	Not described	Not described	Normal	Not described	Only exit the interventricular septal defect
Aorta	Normal	Normal	Right sided arch and descending segment	Coarctation of arch	Truncus arteriosus arising from right ventricle. Arch had 4 branches. Descending segment on left
Other abnormalities	Osseous abnormality of neck	None	Accessory spleen	None	Absent spleen; rudimentary stomach; bent vertebrae; one umbilical artery



Fig. 2



Fig. 3

Fig. Lary (opened). Note sharp edge of mucosa lining line of fusion (× 7). Fig. 3. Posterior aspect of heart and lungs probe ... persistent left superior vena cava and azygos vein marker ... left vena superior and inferior venae cavae.

entire thoracic cavity. Removal of the ... mediastinum revealed empty pleural cavities (Fig. posterior).

The mouth, nasal passages, tongue and pharynx were normal. The larynx was hypoplastic, the aditus laryngis measuring 0.4 cm × 0.25 cm. It was formed normally but shortened aryepiglottic folds curving round the epiglottis to meet posteriorly (Fig. 2). Corniculate tubercles were recognisable on either side of the prominent midline interarytenoid notch. Deep to them lay arytenoid cartilage masses. The depth of the posterior laryngeal wall was 0.1 cm. From this point to the base of the epiglottis, the laryngeal side walls were fused producing a blind pit shallow posteriorly and deep anteriorly. On separating the walls, neither vestibular nor vocal folds were present at the base of the epiglottis (Fig. 2). The thyroid cartilage was normally developed, the larynx lying behind it with its base situated just above the lower border. The epiglottis was very sharply retroflexed in relation to the upper border of the thyroid cartilage. The cricoid cartilage was absent. There was no vestige of trachea, bronchus or pulmonary tissue. The thyroid gland was situated just below the inferior border of the thyroid cartilage; the isthmus was poorly developed. Attached to the lower pole of the right lobe of the thyroid was an accessory cervical thymus measuring 1.2 × 0.8 × 0.5 cm (Fig. 1). The main thymic mass was normally situated, and weighed 5 g. The oesophagus and diaphragm were normal.

The heart weighed 18 g and was scaphoid in shape. The ventricular mass measured 4.5 cm in transverse and 3.0 cm in vertical diameters. The left atrium measured 1.5 cm laterally and 1.8 cm from its posterior aspect to the apex of its appendage. It received no tributaries but blood could flow freely through the interatrial foramen. The right atrium measured 3.0 cm from its superior surface to the tip of the appendage and 2.. cm laterally. Its left lateral margin coincided with that of the common atrioventricular valve (Fig. 3). On its lower anterior wall, lying in a transverse and not an anteroposterior plane, was an oval interatrial foramen measuring 0.7 cm in diameter (indicated behind probe, Fig. 4). Just immediately behind the foramen, a persistent left



Fig. 4. Posterior aspect of heart right atrium opened. Probe in left superior vena cava passes in front of interatrial foramen.

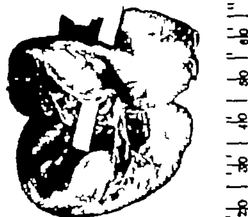


Fig. 5. Interior of heart, posterior aspect. Marker passes through trioventricula valv int left ventricle

superior vena cava and left azygos vein entered the right trium at its anterolateral extremity The lower border of the foramen as formed by thick ridge of muscle which ran semicircularly just bove the trioven tricular valve, from its left to it right lateral margin where it merged with the thinner musculature of the posterior wall of the right atrium. The common bicusped trioventri cular valv measuring 1.3 cm 1 0 cm wa situated more in the outflow tract of the left atrium, its centre being 0 6 cm later l to that of the inferior vena cava. It opened over the centre of the thick interventricular septum which measured 1.6 cm from base t apex (Fig. 5) There was 0.5 cm clearance between the septal apex and the lower edge of the atrioventricular valve cusps allowing free flow of blood across the septum, this being the only outflow from the left ven tricle. The right ventricle was slightly larger than the left, measuring 1 1 cm across the base and 2.0 cm from base t outlet valve ring. Arising from it was a single arterial vessel of wide bore whose orifice was tricuspid measuring 0 9 cm in diameter (Fig. 6) There were right and left anterolateral and posterior cusps. The left coronary artery arose from the posterior sinus, the right from the left anterolateral sinus. There was no pulmonary trunk. From the arch of the trun cus arteriosus arose four vessels, the innominat left common carotid left vert bral



Fig 6 Superior view of heart showing opened truncus with origin of coronary arteries and arch vessels left trium also opened

and left subcl ian arteries. Although it originated w ll to the right of the midline the rch passed obliquely backw rd so that the descend ng segment wa on the left. Owing to the absence of the ductu arterio-sus the left recurrent laryngeal nerve hooked round the aortic arch.

Hist logical sections from the line of f sion of laryngeal ide wall showed a lining mu cosa of ciliated columnar epithel um co ering large numbers of mixed m cous gland embedded in firm connecti tissue in whose depth lay irregular masses of hy line cartil ge. No ther respiratory tissues were present. Examination of the rema ning ti ues d d not reveal any abnormality.

Embryology

Two different pulmonary Anlagen are re cognised an epithelial one which play the main role during the formati period of the organ, and a mesench mal one whose importance increases as the functional stage is approached [6]. The epithelial Anlage ppears as a median entral groove-lik d verticulum of the foregut and is respon sible for the lining epithelium and associated gland of the larynx trachea and bronchi and the respiratory epithelium of the lveoli [7]. The upper portion of the larynx (above the vocal folds) dev lops from the pharyngeal floor i the region of the primitiv glottis the slit opening from the floor of the pha rynx into the trachea [ ]. At the 5 mm st ge primary bronchi ha e started to bud from the caudal end of the laryngo-tracheal groove; these, by growth and branching, produce all subdivisions of the respiratory tree. Just prior to this the aortic arches develop, the first ones during the early part and the ixth (pulmonary) arch t ward the end of the fourth week.

At the 5 mm stage of development (end of the fourth week) the heart consist of three undivided chambers

1 the sinus venosus opening into the right atrial dilatation,
2 the bilaterally dilated trium communi cating by a common atrioventricula ca nal with
3 the primitiv ventricle.

Interventricula septal development has al ready commenced. Atrial partitioning is achieved by d wngrowth, t a slightly later t ge of the septum primum to join the endocardial cushions which have then f sed in the m ddle of the common atrioventri cular canal. At this time the truncu ar teriosus is being d vided into aortic and pul monary trunk by fusion of two prominent longitudinal ingrowths of endocardial lining whose inception occurred prior to the 5 mm tage. These hanges occur in Horizon XVI ovulation age 33 ± 1 day (Streeter [18]). Streeter in a series of papers [16-19], de fined ge groups or developmental horizons in embryos by reference to numerous fea tures or scoring point easily recognisable on sections which he found characteristic for particular lev l of dev lopment. A organ correlation follow a defini in ariable sched le the presence of these point allows of accurat timing of coincident modifications in unrelated organs. The corresponding em bryonic age is calculated by comparison with macaque embryos of known ovulation ge.

Discu ion

Attention ha been drawn to th many cardio-respiratory development occurring ju t before the end of the fourth week of embryonic life (Horizon XII-ovulation age 30 ± 1 day [10]) ince it i felt that about this time the embryo was affected by a lethal factor which interfered with cardio-respiratory development. The ana tomical lesions present can be explained on this basis.

Broncho-pulmonary development cea sed during the 4 mm stage—certainly be fore the groove elongated and produced lung bud (5 mm). This would allow nor mal development of the upper larynx with subsequent formation of arytenoid masses,

whilst preventing that of the lower larynx, trachea and lungs

The cardiac structures affected are the just developing endocardial cushions, longitudinal truncus thickenings and sixth aortic arches. Failure of their development would explain the common atrioventricular valve interventricular septal defect truncus arteriosus and absent pulmonary arteries The septum primum, in the absence of fused endocardial cushions appears to have deviated to the left and become attached to the left lateral wall of the atrioventricular valve so explaining the anomalous transverse position of the interatrial foramen, which, from its location at the most caudal part of the interatrial septum, is a persistent ostium primum. Over-absorption of the initial part of the left subclavian trunk, with separate origin from the aortic arch for the left vertebral artery has produced four arch vessels. This is the commonest form of aortic arch anomaly [3]

Congenital absence of the spleen is rare between it and congenital malformations of the heart and great vessels is an unusually high degree of coexistence Ivemark [9] analysed 69 cases of splenic agenesis and showed that in each case with cardiac anomalies where there was an adequate description of the findings abnormalities of both cono-truncus and atrioventricular (A–V) regions were present. Coexistent with splenic agenesis and the cardiovascular malformations in many cases were anomalies of other organs including liver alimentary tract and lungs each of which bowed a tendency towards symmetry He felt the association was of sufficient frequency to constitut syndrome which he termed asplenia

He showed from study of serial sections of embryos that the early stages of splenic development correspond in embryologic time with certain modelling changes in the heart affecting both cono-truncus and A–V regions and using Streeter s scoring points for defining embryonic age he was able to place the time of appearance of earliest morphological evidence of splenic primordia in Horizon XV–ovulation age 31±1 day [18]. It has been shown that both cardiac and respiratory anomalies in the present case arose in Horizon XII. this implies either that splenic agenesis was a separate isolated event or that its inception is earlier in time than has previously been supposed. Ivemark suggested that histochemical methods would allow of earlier location of splenic primordia, were they possessed of a known specific stainable substance The present case with its anomalies of both cono-truncus and A–V regions exactly similar to those described by him in association with splenic agenesis suggests that the earliest stages of splenic development, incapable of detection by ordinary microscopical methods may occur in Horizon XII

In more than 40% of cases of this syndrome of visceral symmetry there are anomalies of systemic venous drainage the commonest being persistence of the left superior vena cava, in one case this was associated also with a left azygos vein as in the present case In each of Ivemark s cases the left superior vena cava entered the left atrium. Abnormal pulmonary lobation occurs in two thirds of cases of asplenia. The lungs are usually mirror images with most often three more or less complete lobes on each side though in one case 5 lobed lungs were present. In none

of hi cases wa the abnormality a reduc tion. Bilateral absence i of course sym m trical but in view of the gastric hypo pla ia the presence of a w ll developed interventricular septum in an otherwise typical example of persistent truncus arteriosu with splenic agenesi (Type A) and the entry of tl e left superior vena cava into the right atrium features seen in none of Ivemark s cases it is felt that this is not a true example of the syndrome but rather a more complex abnormality whose com ponents are more likely to have arisen as separate malformations linked only by time of origin and causative agent The developing lungs heart tomach and dorsal mesogastrium in an embryo of Horizon XII are very cl sely related spatially and it i not impos ible to conceive of a single factor with only a limited field of action producing related anomalies of this type

In view of the teratogenic effect of Thalidomid in the production of phoco-melia other maternal drugs could perhaps act in thi way Experimentally in foetal rats of vitamin A deficient mothers lung agenesis has been seen in association with various cardiovascular anomalies one of which is bilateral absence of the s xth (pulmonary) aortic arch [21]. The fact that lesions of this type can be produced by a specific dietary deficiency further supports the concept of a ingle aetiological agent in this case its nature however being unknown

The table compares the important find ings in our case with tho e of all pre viou ly described cases of bilateral pul monary agenesis As Claireaux & Ferreira [4] observed in each case including the present one attempts at respiration have been made indicating that initiation of respiratory movement requires the pre-sence of neither pulmonary bronchial nor tracheal tissues

Duplication of the spleen i caused by an arrest cf fusion of the incisures which develop in its surface as early as Horizon XVI Ivemark [9] described examples of cardiac anomalies in association with rudimentary and multiple spleens these were of varied nature and included cases with normal cono-truncus and A V re gion He f lt the explanation for this wa that in hypopla ia and reduplication, or ganogenesis had gone astray at a sl ghtly later stage In this context the accessory spleen present in Turnman & Gardner s case [20] is interesting a cardiac malforma tions also were present though not of cono-truncu or A-V region The arrest of pulmonary development which occurred at a later stage than in the present case can also be placed at about the sam em bryologic time

Both Claireaux & Ferreira [4] and Turn-man & Gardner [20] con idered th cardiac lesions present in their cases arose as a direct consequence of bilateral pulmonary agenesi Study of the cases of Schmit [14] and Allen & Affelbach [1] supports this suggestion in so far that, in their cases also, a pulmonary trunk was present entering the aorta as the ductus arteriosu and they lacked pulmonary veins In the pre sent case the malformation of tomach, spleen, respiratory and cardiovascular system arose at the same stage of em-bryogenesi and it ha been sho n that they are most probably independent having in common only their promoting agent and time of origin It i likely that time and perhaps the intensity of onset of the lethal factor determine the definitive

pattern of anomalies and in this respect, the cardiovascular system has greatest scope for variation. The differences in the cardiovascular anomalies in previously reported cases and in this one, thus may be merely the results of interference with development at slightly later stages and in all cases, the cardiac lesions may arise not as a direct result of pulmonary agenesis but as a separate malformation.

Summary

A case of bilateral pulmonary agenesis and the first of total tracheopulmonary agenesis, is reported. In addition to absence of the lower portion of the larynx, trachea and lungs, there were several cardiac abnormalities, including a common atrioventricular valve, persistent ostium pri mum interventricular septal defect trun cus arteriosus and absence of both pulmonary trunk and arteries. Additional malformations present included a per sistent left superior vena cava left azygos vein, absence of the spleen, gastric hypoplasia and a hemivertebra The embryology of the defects in the present case is discussed with particular reference to their time of onset and causation and the im portant findings compared with those of previously published cases It is suggested that inception of splenic development may occur earlier than has previously been postulated.

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References

1 Allen and Appelbach: Congenital absence of both lungs. *Surg Gynec Obstet 41* 378, 1925.
2. Arey L. B.: *Developmental Anatomy* 6th ed. W B. Saunders Co., Philadelphia, 1954, pp. 260, 331
3. Brash J C.: *Cunningham Textbook of Anatomy* 9th ed., Oxford Univ Press 1951 pp. 831 1251
4. Claireaux, A. E. and Ferreira, H. P. Bilateral pulmonary agenesis. *Arch Dis Child, 33* 364, 1958.
5. Ellis, A. G Congenital absence of lung. *Amer J Med Sci, 154* 33 1917
6. Gruenfeld G. E. and Gray S H. Malformations of the lung. *Arch Path, 31* 392, 1941
7 Hamilton W J Boyd, J D and Mossman H. W *Human Embryology* 3rd ed., Heffer Cambridge 1962, p 290
8. Heerup L. Case of absence of left lung. *Hospitalstidende 70* 1168, 1927
9. Ivemark, B. L Implications of agenesis of the spleen on the pathogenesis of conotruncus anomalies in childhood. *Acta Pædiat Scand, Suppl 104* 1955.
10. Jones, H E. and Howells, C. H. L. Pulmonary agenesis. *Brit Med J II* 1187 1962.
11 Mariconi, P Agenesis of the lung *Dis Chest 41* 232, 1962.
12. Olcott C. T and Dooley S. W Agenesis of lung in an infant. *Amer J Dis Child 65* 776, 1943.
13. Oyamada, A Gasul, B. M. and Holinger, P H Agenesis of the lung *Amer J Dis Child 85* 182, 1953.
14. Schmitt H Ein Fall von vollständiger Agenesie beider Lungen. *Virchows Arch Path Anat 134* 25, 1893
15. Schneider, P in Schwalbe, E.: *Die Morphologie der Missbildungen des Menschen und der Tiere*, Jena, G Fisher 1909–1913, vol. 3, Pt 2, p. 763.
16. Streeter, G. L.: Developmental horizons in human embryos Description of age group XI, 13 to 20 somites, and age group XII, 1 to 29 somites. *Carnegie Contrib Embryol 30* 209, 194_.
17 — Developmental horizons in human embryos. Description of age group XIII, embryos bout 4 t 5 millimeters long, and

age group XIV period of indentation of the lens vesicle. Carnegie Contrib. Embryol. 31 No. 199 pp. ?? 63, 1945.
18 — Developmental horizon in human embryos. Description of age group XV, XVI, XVII and XVIII being the third issue of survey of the Carnegie Collection. Carnegie Contrib. Embryol. 32 No. 11 pp. 131 ?? 1948.
19 — Developmental horizon in human embryos. Description of the age groups XIX, XX, XXI, XXII and XXIII being the fifth issue of survey of the Carnegie Collection. Prepared for publication by C. H. Heuser and G. W. Corner. Carnegie Contrib. Embryol. 34 No. 230 pp. 165-196 1951.
20 Tuy ?? P. L. and G ?? L. W. Bilateral ?? of the lung. Arch. Path. 34 308 1942.
21 Wilson J. G. and Warkany J. Aortic arch and cardiac anomalies in offspring of vitamin A deficient rats. Amer. J. Anat. 85 113, 1952.

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CASE REPORT

Arthrogryposis Multiplex Congenita

A Case of Neurogenic Origin

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Arthrogryposis multiplex congenita (a.m.c.) is characterized by a congenital limitation of movement in a varying number of joints which are retained in the extended or flexed position. At the same time the shoulders are rotated internally and the legs are rotated externally at the hip joints.

Although the disease has been known for a long time its etiology and pathogenesis remain a matter of dispute. The discussion is now concentrated on three main points of view: (1) primary muscular disease ( ) primary neurogenic affection and (3) universal mesenchymal dysplasia

Until 1951 when James [8] collected a total of 240 cases of a.m.c. from the literature, only six autopsies had been described, three of which did not include an examination of the central nervous system. Phocas [9] discovered that the muscular contraction did not seem t be the principal cause of the fixation of the joints, since the joints remained in the fixed position even if the muscles were cut through. This finding was erified by Howard [7] in 1908 on the basis of thorough autopsy At this autopsy no macroscopic lesions were revealed in brain or spinal cord, which were however not examined microscopically Conversely histological examination of the muscles showed fatty degeneration of differentiated muscle fibres, and Howard believed that the disease primarily was a muscular disorder In 1933 Price [10] found degenerative lesions f the cells of the anterior horn and fibrofatty degeneration of the muscles in a patient with a.m.c and he advocated the theory that it is a primary neurogenic affec tion with secondary muscle lesions. In 1947 Brandt [3] described case of a.m.c. with degeneration of the anterior horn cells and a similar affection of the facial nucleus. This thor supported the neurogenic theory On the contrary James [8] concluded in his paper that it must be an embryonal mesen chymal dysplasia which had arisen during the first months of foetal life, and he did not consider the lesions found in the spinal cord to be the primary points of attack of the disorder but supposed that they were co-ordinated with other associated malfor mations, demonstrated by a survey of the literature inter alia mandibular hypoplasia, cleft palat absence of patella, hypospadia, under-developed genitalia and a peculiar subcutaneous tissue. In 1961 Drachman & Banker [5] collected another 14 autopsied cases f a.m.c but in only 10 of these had the central nervous system been examined.

Eight of these cases presented degenerati changes in the terior horn and secondary muscular trophy

Since only few reports are available on autopsied cases including examination of the central nervou system, we will describe a severe case in a child who died at the age of three days

Case Report

The female patient was transferred to the *Children Hospital one hour after birth and* died here at the age of 3½ days No known famil hi tory of diseases. The girl was the only cl ild of young parent Course of preg nancy normal. The mother had had no febrile or exanthematous diseases during preg nancy *Delivery* was normal three weeks before term. Birth weight 2550 g On ad mission the respiration was somewhat frequent and rattling There was intermittent stridor and retractions on the neck, light pallor nd moderate peripheral cyanosis. *When the patient was 22 hours old respira* tion suddenly ceased for about one minut but commenced again after suction. The patient did not react to intubation but from the ge of bout 24 hours she developed recurrent apnoea accompanied by greyish pale colour and cyanosis *in connection with t le* feeding

Physical examination showed the skull to be of normal size the fontanelle was small, the parietal bones were slig tly overlapping The chin was markedly receding It was *difficult to inspect the fauces, since the lower* j w could only be moved so much as to allow the mouth to be opened 1.5 cm. The tongue was rather small and remained in the mouth The child made only weak sucking movements. Nothing abnormal could be heard over *the heart* but *the respiration was harsh* and noisy all over the chest Gradually retractions of the hest developed. The limbs showed pronounced changes in position. The shoulder joints seemed to be normal There was slightly limited extension of both elbows. The hand were held with the wrist i fle ion and could not passively be moved *into the neutral position. The fingers were* held in pronounced flexion t the metacarpophalangeal and inter phalangeal joints and could not passively be straightened The legs lay clasped against the abdomen nd there was but sl ght resistance gainst movement *in the hip joint The* knee joint were hyperextended and could not be flexed normally since there was a resistance in the neutral position. On the contrary they could be hyperextended through 30 Both feet were in the varus position but could be straightened. *The patellae were missing*

Roentgenograms of the limbs revealed infraction of the right femur a slightly posterior and medially angulated fracture being seen between the proximal two thirds and the d stal one third of the right femur *Apart from this no osseous changes or dis*locations were revealed, neither in the pel i nor in the kull bones.

Autopsy (Dr *Charle Johanse* M D Chief Pathologist): The cause of death wa severe haemorrhagic and purulent lobular pneumonia with bilateral fibrinous pleurisy There were no signs of haemorrhage in other organs, in particular not in the central nervous system. No malformations of thoracic or abdominal organs. The entire spinal cord was removed for microscopical examination. *The membranes presented no cha ges.* Cauda was normal. The cut surface was quit soft. At microscopical examination *a slight mye l nation was seen everywhere and the anterior cerebrosp nal f bre were complet ly without myel nation Correspond ngly no motor cells of the anterior horn were seen* No increase in the number of neuroglial cells. In the posterior and lateral horn normal ganglion cells were seen Also here the my lination was considerably reduced corresponding to peripheral nerve tissue At macroscopical examination *striated muscles from the calf were* yellowish brown as it is seen n severe fatty degeneration. Microscopically *the musculature was practically replaced by fatty tissue only a few regions there were sporadic remnants of striated muscles with patchy atrophy*

No inflammatory infiltrations or increased volume of connective tissue. On the contrary skin biopsy showed widespread epidermolysis, considerable and definitely pathological content of acid mucopolysaccharides in the ground substance of the connective tissue, mucinoid oedema and vivid regeneration of connective tissue.

Discussion

In the present case the primary point of attack of the disease seemed to be the spinal cord with total loss of the motor cells of the anterior horn. Correspondingly examination of the muscles revealed a definite neurogenic atrophy characterized by patchy not diffuse atrophy. Other authors found varying degrees of degeneration in the anterior horn cells [3, 5 13]. The latter finding points decisively towards a degeneration and opposes the theory of a primary aplasia. Other authors again found primary myogenic affection at autopsies [.., 5] or muscle biopsies [5 14]. Consequently a distinction is made between myogenic and neurogenic or neuropathic a.m.c., and it is supposed that it will be possible to set up clinical characteristics of both the neurogenic [3 5] and the myogenic form [2].

Clinically our patient presented some of the characteristic symptoms which have been described in neurogenic a.m.c. The affected joints were surrounded by atrophic muscles but the distribution of the muscular lesions had no bearing upon the position of the joints [5]. This is different from the joint deformities in myogenic a.m. where the joints are found to be dislocated in the direction of the strongest muscle [ ]. Unfortunately the periarticular ligament of the affected joints were not examined t autopsy. In the neurogenic form these ligaments are shortened and give rise to the malposition of the joints. Furthermore it is typical of the neurogenic form that the feet are in the equinovarus position [3]. The lower jaw is often underdeveloped [* 6, 8] with a high palate secondary to degeneration of the 5th motor nucleus [5]. The limited movements of the mandibular joints have been described previously as also the missing patella [8, 12] and the tendency to fracture of the limb bones [2 6, 14]. Those symptoms were supposed to be produced by the early muscular weakness, since normal muscular strength in the foetus is of importance for normal skeletal development [5], just as normal development of the joints requires normal foetal movements as early as in the third to fourth month of gestation [1]. Badgley [1] pointed out that the characteristic position of the limbs in a.m.c. with outward rotation of the hip joints and inward rotation of the shoulders, whereby knees and elbows are turned laterally is an early foetal position assumed prior to the normal rotation of the limb buds during the third and fourth months of gestation.

The thickening of the subcutaneous tissue has been described previously by other authors [4 6, 8, 14]. Most frequently the disorder will affect all four limbs [3 5] as in our patient. On the contrary our patient did not present congenital dislocation of the hips, polydactylism, torticollis cerebral or genital malformations, anal atresia, renal aplasia, hydrocephalus, low set ears, pterygium-syndrome or congenital heart disease as described in a number of patients with a.m.c [3 5, 8].

As regards the pathogenesis of the disease many theories have been discus-

sed [11] A relationship between neurogenic a.m.c and Werdnig Hoffmann's disease has been ventilated by several authors [3 10]. In both cases it is a question of degeneration of the cell of the ant rior horn and of the nuclei of the cranial nerves However Werdnig Hoffmann's disease i a progressive disease whereas a.m.c remain stationary after birth In the f rm r disease there are also fasciculations and the contractures occur during the later stages and are less pronounced than in a.m.c However several authors suggest that a.m.c could be an early form of Werdnig Hoffmann's disease or possibly a variant of thi disease Brandt's [3] report of identical twins, of whom one had a.m.c and the other Werdnig Hoffmann's disease supports the theory cf a relationship between the two diseases The clinical course of the disease will depend on the number of motor cells of the anterior horn which are affected, and on how early in foetal life the pathogenic cause sets in, in particular whether it attack before or after development of the pa ive locomotor apparatus.

Summary

A case of arthrogryposi multiplex congenita in a girl who died three days old is described Microscopy of the spinal cord revealed total loss of the motor cell of the anterior horn The characteristics of this neurogenic disorder and it relation to myogenic a.m.c and to Werdnig Hoffmann's disease are discussed

References

1 B DILEY C I Correlation of linic l and anatomical fact leading t conception of the etiology of congenital hip d splasias. *J Bone Joint Surg 25* 503, 1943.

2 B ER, B. Q V ICTOR, M and AD MS, R. D Arthrogryposi multiplex due to congenital muscul dystrophy *Brain, 60* 319 1937

3. BRANDT S A case of arthrogryposis multi ple congenita. *Acta Paediat Scand, 31* 365, 1946/47

4 COMBY J., L amyoplasie congénitale ou raidité articulaire multiple congénitale *Arch Méd E f 36* 171 1933

5. DRACHMAN D B. and BANKER, B. Q Arthrogryposis multiplex congenita. *Arch Neurol (Chicago), 5* 77 1961

6. HEIJENBOK, N I Arthrogryposis multiple congenita. *Monatschr Kinderguese l 10* 67 1941

7 HOWARD R.: A case of congenital defect of the muscular system and it association ith congenital talipes equino- arus. *Proc Roy Soc Med 1* 157 1908.

8. J MES, T Multiple congenital rticula rigidities. *Ed nb Med J 58* 565 1951

9. PROC S, M Flexion congénitale du genou. *Rev Orthop 10* 14 1889

10. PRICE, D B A case of m oplasia congenita, with pathological report *Arch Dis Childh, 8* 343, 1933.

11 ROSSI E. Le syndrome arthromyod splasique congénital. *Hel Paediat Acta,* 62 1947

12. SCHULTZE, K Angeborene multiple Gelenkstarre *Z Orthop Ch 44* 401 1924

13. SWINY RD, C A.: Progressive muscular dystrophy and atrophy and related conditions *Pediat Cl N Amer 7* 703, 1960.

14. TH MSEN F A case of rthrogryposis multiplex congenita. *Acta Paediat Scand 44* 93, 1955.

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CASE REPORT

Congenital Pulmonary Lymphangiectasis

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A rare congenital pulmonary anomaly characterized by lymphangiectasis and lymphatic cysts and known as congenital pulmonary lymphangiectasis has recently received increasing space in the literature The changes were first described by pathologists, but cases have since also been reported by internists [5] roentgenologists [4] and paediatricians [7 8 11 13] The disease is also mentioned in monographs on pulmonary diseases [1 14] So far some ..5 cases have been described. Our case appears to be the first one to be published in Scandinavia.

Report of Case

Male infant the first child of 20-year-old mother and a 21 year-old father The parents were healthy and there were no known pulmonary disorders among relatives. Pregnancy was uneventful. Delivery took place at hospital and was uncomplicated. The child was born t term. Birth weight 3830 g Crownheel length 52 cm. The placenta weighed 690 g and had a normal ppearance Amniotic fluid was of normal amount There was no abnormal bleeding The hild received an Apgar score f 10 points t birth and nothing remarkable was noted at the first general examination by a mid wif At the ge of 8 hours the infant was found t be pale and cyanotic without signs f respiration or heart activity Immediate ttempt t resuscitation proved unsuccessful.

Post mortem Examination

A full-term child, cyanotic without external malformations. The mediastinum showed changes which were interpreted as severe interstitial emphysema with some cysts the size of beans and several smaller ones. The cysts contained a clear fluid. — The lobulation of the lungs was normal. Each of the lobes showed a large number of vesicles, the size of pin heads, which were interpreted macroscopically as interstitial emphysema (Fig 1) The cut surface f the lung showed markedly widened fibrous septa, which d vided the parenchyma int distinct lobules. The lung tissue was generally atelectatic dark red-brown, firm and elastic. The cut surface showed the same picture f widened fibrous septa en losing somewhat bulging, dark red brown lobules. The right lung weighed 33 g and the left 26 g The bronchi contained a moderat amount of foamy mucus but n aspirated material. The other organs were f normal ppearance and showed n malformations. The configuration f the heart was normal. Weight 20 g

Microscopy

The lungs showed irregularly and incompletely expanded alveoli. They were generally hyperaemic with wide vessels and



Fig 1 Heart lung preparation seen from the front Not the thi walled vessels i the mediastinum and the thickened interlobula sept on the surface f the lung Heart down t right

ill area with haemorrhages in the al veoli. Abundant horny debris nd eosinophilic material probably after aspiration f amniotic fluid were foun l in the bronchi and al veol In the widened interlobular septa and particularly subpleurally a generally loose connective tissue cont ined numerous wide spaces lined by thin wall consisting of a single layer of squamous cells (endothelium). Some f these lumina contained small amounts of eosinophilic material (Fig nd 3). — The thymus was f normal structure Around the vessels the mediastinum were thin walled spaces partly lined by endo thelium like cells. These spaces resembled widened lymphatic vessels. The surface of the liver showed a small pale lesion which proved t be a cholangioma. Around the vessels in the liver and in the fat in the renal hilum the lymphatics were moderately widened — Other organs were of normal appearance

Discu ion

Congenital cystic pulmonary changes are often of bronchial origin. That this need not always be the case was stressed by Virchow (1856) [16] who found emphy sema like pulmonary changes with small smooth walled subpleural and interstitial cyst which he took to be widened lym phatic vessel in an otherwise well developed stillborn Bredt (1931) [ ] described a case with similar changes In a report of 3 cases Laurence (1953) [9] used the term congenital pulmonary cysti lymphangiectasis The same year Ciammalvo [6] described a case under the name of congenital lymphangiomatosis of lung form of cystic disease Laurence (19.0) [10] collected 13 cases and used the term congenital pulmonary lymphangiecta is" which has since been adopted by other authors

Analysi of the cases hitherto published shows that pregnancy and delivery had always been normal Half of the mothers were primiparae and the children had been born somewhat before term but often within normal limits. None of the women had been treated with any particular drugs during pregnancy Almost half of the children were stillborn, while th remainder had lived from some hours up to a few days or even weeks [? 4 10–1]. As an exception one child was still ali e at the age of 9 month with constant troublesome dyspnoea and numerous com plicating respiratory tract infections [8]. In that case the diagnosis was made by lung biopsy In children born alive the clinical

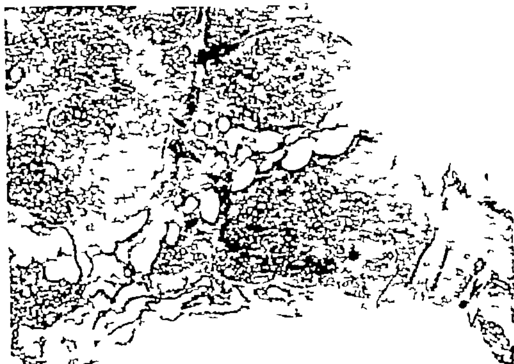


Fig. 2. Section of lower border of lung lobe. Note the system of spaces in the thickened connective tissue subpleurally, interlobularly and around the vessels. Pulmonary parenchyma only slightly aerated. Htx–E. 14

course was always the same: a period of normal respiration for 10 minutes to 1 hour was followed by attacks of coughing, dyspnoea, cyanosis and tachycardia. The condition was diagnosed as congenital heart failure, hyaline membranes or foetal atelectasis. Five cases have been examined roentgenologically [4, 7, 10–1..]. In these cases roentgenography showed fine granular changes over the entire area of the lungs. The picture could not be distinguished from that seen in hyaline membrane disease. All conventional treatment proved unsuccessful.

At necropsy the lungs were found to be large, firm, inelastic and heavy (total weight 50–90 g) with prominent connective tissue septa between the lobules. A subpleural gross network of thin-walled vessels and cysts was common. The cut surface of the lung was of honeycomb appearance with irregularly shaped cysts with smooth shiny walls and up to 5 mm in diameter. These cysts were full of clear fluid. The changes were distributed evenly among all of the lobes. In several cases hypertrophy of the right half of the heart was noted. Hydrothorax was also sometimes seen. Half of the infants had various other malformations of common types e.g. congenital heart disease.

The histological changes in the lungs varied little from case to case. Cystic dilated, thin-walled lymphatic vessels were situated in the subpleural connective tissue and in the interlobular septa as



Fig 3 Closer view of interlobula septum. Dilated cyst with th in connective tissue wall and septa Rest of protein rich material in cystic lumen t bottom A blood filled thin walled vessel in the right margin Ht - E 24

well as around the bronch i and blood vessel The thin walls were built up of loose elastic ti sue and collagen fibres some times with a few mooth muscle cell The inner surf ce of the w ll wa lined by ingle layer of elongated endothelial

The cystic spaces contained mall amount of eosinoph lic material in some cases but in many they were empty The surrounding connective tissue ha been described a loose and embryonal the pulmonary parenchyma i undeveloped and more or less atelectatic No changes have been described in the bronchi while widened lymphatic vessel have been re ported in the lymph nodes at th hilu of the lung

Laurence [10] who used serial section and a projection technique has shown that the cysts and wide vessel were part of a network of thin-walled tortuous duct The distribution of the cysts and the ducts in the connective tissue sul pleurally and in th septa as well as around bronch and blood vessel corresponded to that of the normal lymph vessel Th endothelium like layer lining the wall distingui hed the actual cysts from bronchiogenic cysts, which are lined by cylindrical bronchial or respiratory epithelium Laurence stressed that bronchiogenic cyst are in connection with the air ways that they are filled with air and are not related to the blood vessels He also stated that lymphatic cyst enclose a protein-containing fluid that they are not in communication with the air ways and that they are situated close to blood vessel

Several theories of the development of pulmonary lymphangiectasis have been suggested. The most widely accepted theory i that put forward by Laurence [9], according to which lymphangiecta is should be regarded as a developmental

anomaly The lymph vessels grow into the pulmonary process during the 9th week of foetal life and during the fourteenth week they form wide lymph trunks in the connective tissue which divides the parenchyma of the lung into distinct lobules Normally this lobulation is less distinct after the twentieth week of foetal life when the connective tissue decreases and the lymph vessels become thinner in relation to the parenchyma of the lung According to this theory lymphangiectasis is due to the lungs after the sixteenth week of foetal life continuing to grow without the normal regression of the connective tissue elements.

The above-mentioned theory lends support to Virchow's theory from 1856 that cystic changes in the lungs may occasionally be of lymphatic origin. The pulmonary changes are said to be comparable with congenital lymphangiectasis in e.g a limb. Owing to their mass they have an unfavourable effect on the normal respiratory function of the lungs

Summary

A typical case of congenital pulmonary lymphangiectasis is described and compared with earlier published cases. It is a rare malformation, only about 25 cases hitherto being described. The clinical picture in liveborn infants is characterized by severe respiratory distress appearing a short time after a delivery

References

1. Avery M. E.: *The Lung and its Disorders in the Newborn Infant.* W B Saunders Comp. Philadelphia & London, 1964.
2. Bargt H.: Lymphangiectasis pulmonum congenita. *Virchows Archiv 321* 817 1952.
3. Brucevich, S., Tech Poulsen P and Vestergdal, J Cysts of the lung in infants and children. *Acta Paediat Scand, 49* 30 1960.
4. Carter, R. W and Vaughan H. M.: Congenital pulmonary lymphangiectasis. *Amer J Roentgen, 86* 576, 1961
5. Frank, J and Piper, P G Congenital pulmonary cystic lymphangiectasis. *J A M A 171* 1094, 1959
6. Giammalvo, J T Congenital lymphangiomatosis of lung: Form of cystic disease Report of case with autopsy findings. *Lab Invest 4* 450 1955
7. Heckmann G Kohn R. and Lévy-Solacy J A propos d'un cas de lymphangiectasies pulmonaires congénitales. *Arch Franç Pédiat 17* 1012, 1960
8. Jue, K., S. N Webster, I and Brando, J L.: Congenital dilatation of the pulmonary lymphatics. *Pediatrics 41* 416, 1963.
9. Laurence, K. M. Congenital pulmonary cystic lymphangiectasis. *J Path Bact, 70* 325 1955.
10. — Congenital pulmonary lymphangiectasis. *J Clin Path, 12* 62, 1959
11. Le Tan Vinh, Cochard A. M. and Vu Trieu Dong: Lymphangiectasies pulmonaires congénitales et lymphangite pleuro-pulmonaire cancéreuse métastatique de l'enfant *Arch Franç Pédiat 21* 163, 1964
12. Maidman L. and Barnett R N.:Congenital dilatation of pulmonary lymphatics. *Arch Path, 64* 104, 1957
13. Sacrez, R., Lévy J M., Korn, R. and Paira, M.: Lymphangiectasies pulmonaires congénitales avec fibroélastose de l'endocarde. *Arch Franç Pédiat, 17* 1244, 1960.
14. Spencer, H.: *Pathology of the Lung* Pergamon Press Oxford, London, New York, Paris, 1962.
15. Virchow R. *Gesammelte Abhandlungen zur wissenschaftlichen Medizin,* S. 982. Meidinger Sohn & Comp. Frankfurt a. M., 1856

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PROCEEDINGS OF PEDIATRIC SOCIETIES

The Dani h Pediatric Society

Meeting Sept 0 1961

J Oster: Rare Diseases in a Provincial Pediatric Department

H I Dygge & Marga eta Mikkelsen Partial l letion of the hort arm of a chromosome in th 4 5 group (Dem r)[1]

E traordinary general meeting concerning proposals for new specialist regulations

Meeting Oct 14 1934

Ordinary gener l meeting which included continued d scussion of the new special st regulations

Meeting Nov 18 1934

F Hahnemann Demon tration: (A) A Pa tient with Oxalosis. (B) A P tient with Lowe s syndrome

Mette Hertz: Children i an Infants H me

A review wa undert ken of the hildren lmitted to Arhus M nicipal Home for t during the year 1ax 1961 31 viii

The material comprised 118 i ildren from 10? families (57 married parents, 43 single mothers nd two single f thers) These children could be subdi ided into four groups.

Group 1 53 children and parent presenting n striking feat res.

Group ? 27 ch ldren one of whose parent ppeared sligi tly bnormal and where con tact w th the child was poor et

Group 3 33 children who frequently pre sented psychic symptoms and whose parent were markedly problematical and possibly known to socio-medical and w lfare institu tion etc

Group 4 F ve children inst tutionalized on account of chronic illness

The verage durations of institutionaliza tion for the four groups were: 36, 119 143 nd 213 days, respect vely

It w characteristic for the parent of the children in Groups 1 3 a compared w th the erage population that many were un married, that social standard were low housing conditions poor and contact with w lfare authorities and maternal assistance thorities had been more frequent This latter feat re wa increas gly marked from Group 1 to Group 3.

Co-operation between inst tutions nd dividual who know the problem f milies, social ad isory assistance t initiat contact with nd to encourage the parents, and obser tion by child psych atrist and ps ho logist hav been in aluable in the treatment of these children and this work should be extended even f rther

Sv Hein ld Social Paediatrics

Social paediatrics is not special branch of paediatrics nor a sub-speciality but rather a general concept which is, or should be the foundation of the ind vidual branches of medical knowledge The subject of the pract tioner work today is the ind idual

[1] Published in *Arch Dis Child 40* 82, 1965.

in his environment. The hypothesis may be propounded *that disease is a function of the social structure of society*.

Historically the years around 1600 to 1700 may be considered as the anatomical period which was succeeded by the infective-pathological period and the biochemical period at the conclusion of the previous century and at the beginning of the present century. The period at the middle of this century might be termed the psychiatric-psychological period. The time is now ripe for the commencement of a sociological period.

It is known with certainty that the surroundings or the environment alone can modify even genetically determined diseases (e.g. pneumonia, epilepsy, obesity). Concerning many familial characteristics, it may be stated that not only are they inherited in the genes but also in the traditions. Children with persistent behaviour disturbances remain, as a rule, smaller than children who have no such disturbances. The commencement of a streptococcal infection, despite its undoubtedly contagious nature, appears to be definitely related to family crises (accidents, illness and death, divorce, unemployment etc.)

New perspectives in medical knowledge in the sociological period are required. Retiring behind the "last organic bastions" is useless. Instead, the infinite unexplored marshes which surround the entire organic diagnostic fortress should be put to the plough, irrigated and cultured. This is the object of research in social paediatrics.

DISCUSSION

P. Plum. I agree with many of the points of view expressed by the speaker. The factors which influence the development of a child are still too little known. It is important that the medical profession faces these problems on a biological, scientific level and not from a political point of view.

Meeting Dec. 1., 1964

Meeting with ladies in Domus medica.

C. Friderichsen. A Review of Pediatrics during the Past 50 Years.

Meeting Jan. 13 1935

Aase Nygaard. Ganglioneuroma as the Cause of Chronic Diarrhoea with Hypokalaemia.

A one-year-old boy who had developed normally and who had previously had a tendency to constipation was admitted because of diarrhoea of two months duration. On admission, the child was slightly dehydrated and markedly hypotonic with muscular wasting. Hypokalaemia (1.6 mEq/l) was demonstrated. The patient improved considerably following therapy with fluids and electrolytes. The subsequent course of the disease was dominated by numerous bulky watery stools, massive fluid requirements and electrolyte instability particularly insofar as potassium was concerned.

Coeliac disease and gastro-intestinal allergy were excluded on account of the normal responses to tolerance tests and the lack of response to elimination diets. Secondary disaccharid intolerance was excluded as the faeces were alkaline and did not contain reducing substances. Radiological investigation of the colon and normal rectoscopic findings excluded ulcerative colitis and megacolon complicated by enterocolitis.

The excretion of catecholamines and vanillic mandelic acid in the urine was at the upper limit of normal (83–126–111 μg/24 hours and 7.8 mg/24 hours), and the suspected neurogenic tumour was confirmed radiologically by demonstration of a right-sided suprarenal tumour. The tumour was removed. It weighed 32 g and microscopic

examination revealed it to be a suprarenal ganglioneuroma without histological evidence of malignancy

The diarrhoea ceased immediat ly an l the post-operative course was ncomplicated nd the ch'ld has developed normally since

A. Hobolth a d Margareta Mikkelsen: Mal die de Cri du Chat

This syndrome is due to partial deletion of the short arm of a chromosome no 5. The most marked feature of the syndrome i a prolonged whining cry which ha been compared to a kitten s meowing

A case of this syndrome is reported which was diagnosed in a six-day-old girl by the typical cry and the typical appearance which comprised a round face hypertelorism, epicanthus, slanting "anti mongolic eyes strabismus, hypoplastic receding lower jaw and transverse creases in the palms.

Cytological investigations revealed normal sex chromatin and in all of the cells investi gated partial deletion of the short arm of a hromosome no 5 was found The prog nosis is poor both for ment l development and for survival

J Malholm Hansen A Case of Hypercal caemi in a Girl Aged Eight Months

Following a review of the causes of hyper calcaemia in children a case in a girl aged eight months is reported The child was ad mitted on account of vomiting constipation and f il re to thrive On admission, she was weak and dehydrated. Hypercalcaemia (se rum calcium 1.... mg/100 (ml) was demon strated and the symptoms persisted fol lowing a diet poor in both calcium nd it min D for a month After cortisone therapy for a few days, the serum calcium became normal and linical improvement occurred.

Following limited medication with ita min D the hypercalcaemia recurred and the clinical cond tion deteriorated S l phate therapy was attempted w thout effect while renewed steroid therapy had a striking effect A rise in the alkaline phosphatases was accentuated by administration of vita min D

The diagnosis of idiopathic infantile hyper calcaemia ppears probable and the symp tomatology etiology and treatment are liscussed

DISCUSSION

\ Hobolth In connection with the pre ceding lecture I can report a case of idio pathic infantile hypercalcaemia of the severe type (Fanconi-Schlesinger type)

A female infant nearly one year of age was admitted to the Pediatric Department of Århus Municipal Hospital in 1951 with anorexia, vomiting, constipation and f ilure to thrive On examination she was mentally retarded and also showed retardation of linear growth and weight Her facies were characteristic: a broad bridge of the nose protrusion of both lips especially the lower lip and a receding chin. Though pruritic she w hypotonic with increased deep ten don reflexes. She wa hypertensive and cardiac murmurs were noted on auscult tion. X ray of long bones revealed hyper mineralization. Slight pulmonary stenosis was demonstrated by angiocardiography The most striking chemical abnormalities were a serum clacium of 15.7 mg/100 ml and a urinary calcium of 20.7 mg/100 ml. In add tion serum cholesterol was at the upper limit of normal and hypoproteinaemia was present with raised α_2-globulin.

Administration of thyro d (Thyroidin DAK (R)) in doses of 60 mg daily for month resulted in a slight increase in the serum calcium.

Administration of sodium phytat in doses of 500 mg three times daily was w th out effect after treatment for two and a half months After a supplement of pred nisone 5 mg three t mes daily for three days, the serum calcium fell t just below 12 mg/ 100 ml. After therapy with sodium phytat for 14 months and prednisone for 13 months, all special treatment was withdrawn and the symptoms of hypercalcaemia did not recur

Two years after the cessation of treatment the bones showed normal calcium content

the heart was of normal size and no murmurs were heard. The patient is thriving normally but the appearance is still striking and the child is mentally retarded.

On the basis of the two case reports and a survey of the literature it is concluded that it is difficult to establish a sharp limit between slight and severe forms of idiopathic hypercalcaemia.

N Hebelth Albumin Therapy in Premature Infants as Prophylaxis for the Respiratory Distress Syndrome

Very promising results of treatment were previously reported in discussion in the society Subsequent investigations with treated and untreated control groups have not confirmed the favourable effect of albumin therapy (*Acta Paediat Scand* Suppl. *159* 60 1964)

DISCUSSION

T Iversen Zachau-Christiansen and I did not find any reduction in the morbidity or mortality from the respiratory distress syndrome in infants treated with albumin compared with an untreated control group either (*Acta Paediat Scand* Suppl. *159* 53, 1964).

P Ranlov and O Siggaard-Andersen Late Metabolic Acidosis in Premature Infants

Employing Astrup micro-method total of 632 analyses of the acid-base status were undert ken in an attempt to follow the variations in the parameters in 55 "healthy" premature infant during the first four weeks of life

Late metabolic acidosis could be demonstrated in 42 per cent of these infants. The acidosis commenced, as a rule during the third or fourth day of life, reached a maximum by the ninth or tenth day and, by and large disappeared during the third week of lif The acidosis was uncompensated. It incidence increased the lower the birth weight had been and it was most pronounced in the lowest weight group. The acidosis was not accompanied by any striking clinical symptoms but appeared to be correlated with a certain stagnation in the weight curve It is probable that very protein-rich diet played a certain role but this could not be proved.

The connection between met bolic acidosis and low birth weight revealed in the present material implies that this lat met bolic acidosis must be considered to have more than mere academic interest despite the absence of clinical symptoms.

DISCUSSION

P Kildeberg A direct comparison of this study on "lat metabolic acidosis and that reported by me is difficult because I selected cases showing pronounced acidosis for a closer study rather than attempting a systematic pproach (*Acta Paediat Scand* *53* 517 1964). The diagnostic criteria adopted by Ranlov and Siggaard Andersen as well as the relatively high protein intake of their patients also allow for rather wide ariations in the pparent prevalence of this type of acidosis. On several points our results are in some agreement on others not. In particular I noted that very few of the prematures studied by Ranlov and Siggaard Andersen showed metabolic acidosis of a degree comparable to the low "base excess values of the infants reported on by me. The reason for this is not clear

Lise Wagner Measurement of Blood Pressure in Premature Infants

None of the methods for measurement of blood pressure are suit ble for use in newly born infant and premature infants as the registration of the pulse by palpation, auscult tion and by the flush method gi uncertain results. Registration by means of photo-cell oscillometry was described in 1961 and was found to be well suited for this purpose The method wa described and demonstrated. The preliminary results p pear to demonstrat that the verage blood pressure during the first 24 hours is rela tively independent of the weight group to

which the infant belongs but the blood pressure was found to increase throughout the first weeks of life so that the extrauterine age rather than the size of the infant seems to determine the blood pressure in a given premature infant. In a limited material of infants who died from respiratory distress, the blood pressures were found to be lower. The numerical results are still too limited for statistical analysis.

Meeting Feb 10 1965

A Prader (Zürich): Intestinal Disaccharidase Deficiencies

Meeting Feb 24 1965

J Krangelbock and J Senstius Hypsarrhythmia following Triple Vaccination

Three cases of hypsarrhythmia in infants are reported. These cases were admitted within a period of four months to the Central Hospital in Nykoping F lster. Since the opening of this hospital seven years ago, only two cases of hypsarrhythmia had previously been observed. Both of these were in infants who had been exposed to perinatal brain injury.

The three patients reported had the following features in common: normal pregnancy and delivery and normal development until the age of over five months.

The first patient developed an illness resembling encephalitis in direct association with the first triple vaccine polio injection. Her development ceased but the second and third injections of triple vaccine polio vaccine were administered, nevertheless, according to the usual schedule. On admission, at the age of 8½ months, she had characteristic attacks of hypsarrhythmia in series. The EEG showed a typical hypsarrhythmia pattern and air-encephalography revealed severe atrophy of the brain of mixed cortical and ventricular type. The CSF was normal.

The second patient was 6½ months of age on admission. He had been vaccinated for the first time one month previously. There had not been any immediate reaction to the triple vaccine but 13 days later the first attacks of infantile spasms were observed and these were followed by weakness and remoteness. Over two weeks later he was admitted on account of increasing frequency of the attacks. On admission, he was irritable and cried frequently. Several independent attacks were observed. The EEG showed a typical hypsarrhythmia pattern. After cortisone therapy for a week, he improved considerably and the EEG showed considerable improvement. During continued steroid therapy which was gradually supplemented with phenemal, deterioration occurred with severe EEG changes and air encephalography now revealed moderate atrophy of the brain.

The third patient was 7½ months old on admission. She was vaccinated with triple vaccine at exactly five and six months and received the third injection at over seven months. Three weeks after the second vaccination the first attack of hypsarrhythmia was observed. After the third injection of vaccine the attacks increased in frequency and led to admission. On admission, the infant was cross and irritable. The EEG showed a characteristic hypsarrhythmia pattern. After steroid therapy for one week, the child improved mentally: she became happy and played and was free from attacks. She could be discharged after six weeks in good condition. The steroid therapy was continued for a total of nearly three months. Follow up examination one month after the conclusion of treatment revealed normal EEG and, clinically the child was found to be healthy with normal motor and mental functions.

In approximately 50 per cent of all the cases, the etiology of hypsarrhythmia is unknown. From the material presented here

there seems t be no doubt that the first patient developed encephalopathy in direct connection with triple vaccination while as regards the second and third patients, it can merely be stated that there is no other etiological explanation and that there is, therefore reason to presume that there is a connection between the hypsarrhythmia and the triple vaccination.

Hypsarrhythmia following administration of pertussis and triple vaccines, respectively has been reported by Baird & Borofsky (1957), from Sweden by Justus Strom (1959) and from Norway by K. W Wefring (1960) but this complication f vaccination has not hithert been reported in Denmark.

DISCUSSION

P W Brandrup Out of a total of seven children with infantile spasms admitted t the Pediatric Department in The Copenhagen County Hospital in Gentoft during recent years, five had either not been vaccinated t all or had another clear explanation for the spasms. One child a premature male infant aged four months, had received three injections of whooping cough vaccine, the last of which was three weeks before the commencement of the symptoms. The condition progressed despit steroid therapy and the child is now taken care of under the Mental Deficiency Act

Another child developed spasms in immediat connection with the second injection of triple vaccine at the age of eight months. Administration of corticotropin produced a dramatic clinical effect and the EEG returned t normal in the course of a week.

P Plum It is very important to lucidat this question further One method is to ask all Pediatric Departments to report on their materials of infantile spasms.

Sv Tulinius The Danish N tional Serum Institut at one time warned against the use of triple vaccine The reactions are probably due to the whooping cough accine

P Plum It is probably wiser to w thhold whooping cough accina ion in infant under the age of one year

C. Sand Jespersen, J Littauer and U Sagild:
Measles during Pregnancy as th Cause f Foetal Death and Congenital Deformities

The first epidemics of measles occurred in Godthåb, Jakobshavn Umanak and Angmagssalik in Greenland during the years 1954 to 1962. The result of a retrospective investigation comprising 155 pregnant women, infected during these epidemics, is presented. The incidence of foetal death and the occurrence of congenital deformities, the incidence of prematurity and the infantile mortality are illustrated.

Thirty-three women developed measles during the first trimester and a third of these pregnancies ended with foet l death (abortion) 14% of the live-born infant had congenital deformities, 18% were premature and the infantile mortality was 23%. Only half of the embryos affected during the first trimester survived the age of one year

Fifty four women were affected during the second trimester and 6% of these pregnancies ended as abortions or stillbirths; no cases of congenital deformities occurred, 15% of the live-born infants were premature and 8% died prior to the age of one year

Sixty-eight women were found to have been infected during the third trimester All of these gave birth to living infants, one of which had a congenital deformity Only 7% of the infants were premature and the infantile mortality was 9% in this group

On comparison with prospective investigations of 103 pregnant women infected with measles (Manson, 1960) and 294 cases of maternal German measles (Siegel & Greenberg 1960) it is considered that the conclusion may be drawn that infection with measles in the first trimester of pregnancy involves a risk for the foetus comparable with that previously recognized in infection with the rubeola virus.

DISCUSSION

H Andersen Considered that control materials were required.

P Plum. The result of the investigation is alarming Hitherto, only rubeola was con-

sidered with certainty to be the cause of congenital deformities. The material itself seems to provide adequate control material

P E Christense In ou work on the first epidemic of measles in Greenland in 1951 (*Ugr l. Iæg* 1953) an increased in cidence of bortions was similarly encoun tered together with an increased number of stillbirths, but no increased incidence of congenital deformities.

F Buchthal and B Friis Hansen Focal EEG Changes in an Infant with Acut Salt Poisoning

On a previous occasion, a case of salt poisoning in an infant was demonstrated to the Society (Hansted, 1959) but the present case is of interest because focal EEG changes which disappeared in the course of 4 hours were encountered.

An infant aged five weeks was admitted two days fter examination in a hild wel fare centre because the f ther had acciden tally p t salt into a feed instead of sugar The infant had vomited t home but wa not affected in any other w y on admission. The serum sod um was found to be 176 mEq/l serum chloride 143 mEq/l and bi carbonat 15.6 mEq/l. The infant had lost 300 g in weight By mistake 600 ml pure glucose solution was administered in the course of the night and the child was sent for EEG recording the following morning The EEG showed cont nuous paroxysmal act vity over the right hemisphere with large spikes and slow waves. Following the EEG recording the infant suddenly developed n ttack of generalized spasms lasting for an hour despit adm nistration of 50% glucose and calcium lævulat intravenously The case was interpreted as acut water intoxication as the result of too rapid re hydration following salt int xication. The serum sodium fell to 146 mEq/l during the convulsion. F rther s pport e evidence is that the infant had gained 250 g in weight in the course of the night The blood sugar and the serum calcium were both normal during the convulsion. Following the con vulsion the infant improved rapidly and during the subsequent days, normal feeds were gradually introduced and the infant recovered completely Daily EEG recordings howed normal findings and follow-up examination three and four months after discharge revealed completely normal find ings. The case is of i terest because transient focal EEG changes have not previously been described in cases of w ter intoxication.

DISCUSSION

J Kringelbach Is it possible that the infant lay on the right side and leveloped oedema?

B Friis Hansen did not think so.

I W Broestrup thought that he had seen localized EEG changes following post operative over hydration.

A Wennevold E. Sandøe and J C. Melchior Inderal in the Adams-St kes Syndrome in Children

Two girls aged 8 and 13 years, had, for several years hibited ttacks of loss of consciousness on exertion on account of rapid cardiac rrhythmia. Neither of them had other cardiac symptoms and, part from sinus brad cardia in the older girl, the findings on stethoscopy radiography of the thorax and ECG at rest were all normal.

As there were no subjecti sensations of cardiac rrhythmia prior to the attack and the EEG was slightly abnormal, confusion with epilepsy was understandable and the younger girl had been treated for epilepsy for six months. The older girl developed her first ttack some months after a severe at tack of measles and as there wa bradycardia at rest attention was primarily focused on the heart in this case. In both cases, the diagnoses were est blished by ECG recordings during or immediately after severe xertion when long salvoes of mult focal ventricular extrasystoles were observed.

On the assumption that irritability of the myocardium was responsible and that the sympathetic nervous system increases the irritability of the myocardium, treatment

was instituted with propranolol (Inderal) As an adrenergic blocking agent this drug blocks the beta receptors in the myocardium. The effects of the propranolol therapy were followed in both cases by means of ECG recordings on exertion. It did not prove possible t suppress the arrhythmias completely but both patients could carry out more strenuous exercise before rrhythmia developed and, similarly sinus rhythm was re-established more rapidly after discontinuing exertion.

Both of the patients were followed up for four months. The younger girl became completely symptom free The older girl had two brief Adams-St kes ttacks but si multaneously she became so fatigued that Inderal has been temporarily withdrawn.

DISCUSSION

B Friis Hansen Did muscular spasms occur during the attacks?

E Sandøe. There are probably number of similar cases among patients undergoing treatment for epilepsy The clinical picture varies and the only characteristic feature is that the attacks occur on exertion.

B Zachau-Christiansen and Helge Pedersen
Foetal Haemoglobin in Premature Infants

As early as the tenth week of foet l life, HbA has been found in the foetal blood but HbF appears to be maintained t a level of about 90%, as determined by the alkaline denaturation methods (Betke Singer) until found bout the normal duration of pregnancy (Walker & Turnbull) HbF or rather the logarithm of % HbF/birth weight is, however according to Brody a good expression of the duration of pregnancy It was further demonstrated (Jonxis & Brody) that infants suffering from neonatal haemolytic disease had low HbF alues.

Brody and his co-workers have demonstrated that the maximum serum bilirubin increases with increasing HbF content in non-sensitized infants. Further t has been demonstrated that even the total quantity of HbF in infant is more or less constant from infant t infant.

Reliable methods of demonstrating HbF in small quantities (Krut ffersen s method among others) have attracted interest in recent years. Normally less than 2–4% HbF is found in adults. If the figure is raised in pregnant women, this may indicate foetal death. Finally it should be mentioned that HbF may be present in increased quantities in a series of haemoglobinopathies probably as a compensatory phenomenon

In the Children Hospital, Fuglebakken, Singer's method has been modified so that it can be employed at routine Hb determinations. This is undertaken by photometric determination with 541 mμ of the Hb content in 7 ml 0 1% Na_2CO solution to which 25 μl capillary blood is added.

To 3 ml of the bovementioned Hb solution 10 ml 10% N OH is dded and the mixture is shaken for 60 seconds. Alkaline denaturation is then stopped by 2.9 ml of a saturated solution of ammonium sulphat to which 1 ml 10 N HCl is added per 200 ml After repeated shaking for 60 seconds, the precipitated protein is separated by filtration and the HbF is found from extinction of the filtrat t 541 mμ.

Employing this method, the HbF in normal newly born mature infants was found to be 54±10% ($n = 10$) and in premature infants 65±6% ($n = 19$) These figures are lower than those found by Betke method and at the lower limit of those found by Singer's method employing only weak Hb dilutions. The differences may be due to errors in Betke method on account of extrapolation and loss of Hb by filtration in Singer method This loss is accentuated in weak dilutions. Further in Singer's method, the total Hb is measured in alkaline solution and the HbF in acid solution.

Forty nine premature infants were f l lowed up during the first year of life. The decrease in the relati e HbF content followed the course in mature infants as found by Zipursky *et al.*, with the exception that the values were approximately 15% higher (correction was undertaken for systemic difference between the methods employed). After 24 weeks, less than 5% was found.

then bout 14 days after beginning Olac and, in some cases, again 14 days later

A control group of 12 patients received breast milk exclusively until they weighed 2700–2900 g after which they received a 50 milk mixture Blood samples were taken at times corresponding to the above

Finally in 10 patients of the same age and weight group the serum lipids were measured on diet of 50% milk mixture after the infants had received this diet for at least one week.

On investigation of the total lipids and phospholipids, slightly lower values were found in the infants on Olac than in those on breast milk but the difference is not signi ficant N differences were observed between the infants receiving the milk mixture and Olac.

The serum cholesterol values were found t be significantly lower in the infants receiving Olac (101.2 ± 18.4 mg/100 ml) as compared with breast milk (120.2 ± 24 1 mg/100 ml.) A distinct decrease was bserved on transition from breast milk t Olac This was not due to the fact that the infants were older as only small and insignificant fall was bserved in the children who continued to receive breast milk. The infants receiving the milk mixture showed the same blood cholesterol values as those receiving breast milk, viz., 124 ± 13.8 mg/100 ml.

These findings are in agreement with the results of previous investigations in mature infants. Where premature infants are con cerned, very few investigations are vailable mainly by Gyorgy *et al* who found lower serum cholesterol and serum lipid values on diets with vegetable fats with high un saturated fatty acid contents as compared with diets containing animal fat The pre sent results are in good agreement with this. It is singular that breast milk and cow milk give the same alues for the serum cholesterol level although the content of un saturated fatty acid is considerably lower in cow milk than in breast milk. The bservation that Olac result in significantly lower values for serum cholesterol than breast milk although it contains the same quantity and type f unsaturated fatty acid as breast milk, cannot be explained.

It is impossible to stat whether it is healthier for the infant t ha a low serum cholesterol level. It would be equally justi fied to suppose that a low serum cholesterol had harmful effects and it is probably too early to take measures to prevent arteriosclerosis.

D SCUSSION

B Zachau-Christiansen. The tendency t lower lipid values in infants receiving the experimental preparation than in those receiving breast milk and th relatively wide scatter observed in the total lipids in the serum could perhaps be explained by the fact that the preparation concerned, when emulsified, had a much greater difference in the diameters of f t globules than is observed naturally in breast milk and cow milk. The preparation was available in two portions, in the second of which an attempt had been made to reduce the size of the fat globules

In the Children Hospit l, Fuglebakken, where we have also worked with this hu manized milk preparation, we had the im pression that it too frequently produced fatty stools with thrice the fat excretion as breast milk. W ttributed this phenomenon t the content of large fat globules. W also made the bservation that the size of the fat particles may be of significance for the ppearance of the stools even with breast milk. Frozen breast milk, fter thawing, also shows great difference in the size of the fat particles and produces looser and apparently more fatty stools than fresh breast milk.

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Studies on the Streptococcus Group A Infection

The Carrier State and Latent Infection among Childen

by P MOZZICONACCI, J GERBEAUX,[2] A. BACULARD [2] CL. GERBEAUX,[3] R. CARAVANO [1,3]

with the technical assistance of

Mrs G FOURNIER, J LEMAIRE and C. ROTH

It is a general observation that the biology of infections by commonplace microorganisms has changed considerably in countries where the standard of hygiene has reached a certain level. As far as infection by haemolytic streptococcus is concerned, several of the chapters which were found under this title in pathology textbooks of some decades ago have lost very much of their significance. Suppurative fevers, puerperal sepsis, erysipelas, lymphangitis and in some countries, even scarlet fever have become very rare.

This evolution of the pathology of infections as related to Group A streptococcus seems to be due mainly to a biological modification of the relationship between the host and the parasite for the latter remains extremely widespread. Without doubt the introduction of active antiinfective therapeutic agents has contributed greatly to this change but a contradiction still persists. Antibiotic therapy of well-known streptococcal infections guarantees the prevention of and an efficacious prophylaxis against serious complications (among the most important of which is rheumatic fever) yet, these keep even in countries possessing a relatively high standard of living, by no means a negligible incidence [12]. This might be a residue of the period before the general introduction of prophylaxis, which is relatively recent; or it could be caused by the fact that many common streptococcal infections are undoubtedly treated by antibiotics from the beginning with insignificant doses administered and even the exact diagnosis not having been clearly established, but it could also be the result of the persistence of endemic streptococci which, while being clinically attenuated, would nevertheless provoke as much damage as would a more acute infection.

This is likely why investigations concerning the epidemiology of minor infections due to Group A streptococcus and the carrier state of this microorganism in

This study has been supported by the Society for Help to Rheumatic and Cardiac Children (SESERAC) and the National Institute of Health and Med. Research. Dr Caravano has been awarded fellowship from the Helen Hay Whitney Foundation, New York, N.Y.

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[2] Hôpital Léon Bernard, Brévannes, S. & O., France.

[3] National Institute of Health and Medical Research.

TABLE 1 *Distribution of ages of the children examined*

(Age in years and months)

				7/11								
				7/11								
				7/10	8/10							
				7/10	8/10							
				7/9	8/10							
			6/11	7/9	8/9	9/11	10/11					
			6/11	7/9	8/9	9/9	10/10					
			6/11	7/8	8/8	9/9	10/10					
			6/11	7/6	8/8	9/8	10/7					
			6/10	7/6	8/6	9/8	10/7					
			6/9	7/6	8/6	9/8	10/6					
		5/11	6/8	7/5	8/5	9/7	10/5		12/11			
		5/10	6/8	7/4	8/2	9/7	10/5		12/9			
		5/9	6/8	7/4	8/2	9/5	10/4		17/7			
		5/8	6/6	7/3	8/1	9/4	10/3		12/7			
		5/7	6/5	7/3	8/1	9/4	10/2		12/7			
		5/5	6/2	7/3	8/1	9/3	10/1		12/6		14/9	
		5/4	6/2	7/2	8/1	9	10	11/9	12/6		14/4	
	4/10	5	6/2	7/1	8	9	10	11/8	12/6	13/10	14/3	
3/4	4/1	5	6/1	7/1	8	9	10	11/3	12/5	13/3	14/1	17/4

of these children were sufficiently observed to be included in the complete clinical study bacteriological and serological, which was therefore carried out on a restricted group of 88 of these subjects.

Results

Results of swabbing of the throat

Among 114 children retained for this part of the study the distribution is more

TABLE 2 *Length of observation* (in months)

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
Order number of subjects.					171							
					170							
					169							
					163							
					166			148				
					165	165	157	143				
					148	161	156	142				
					108	160	154	141				
					86	158	152	139				
					78	150	148	136				
					70	147	135	131	140	138		
		178			59	130	134	124	137	133		
		177		174	53	107	132	*93	123	130		
	176	144	175	173	35	89	129	*92	122	128		
	172	114	143	105	34	33	80	91	121	126		
	156	106	100	97	18	30	76	83	119	118		105
	112	103	88	17	16	27	52	78	116	110		101
	69	39	71	14	15	23	12	40	113	65	111	*99
164	8	22	10	1	7	6	2	45	*94	81	104	*96

* Subjects belonging to the limited group.

or less equal between non-carriers, 60 (5 ,.) and carriers 54 (48 %)

Children from North Africa seem to be more frequently carriers (18 of them among the carriers, 8 among the non-carriers) but statistically this difference is not significant ($\chi^2 = 3.69$ with 1 degree of freedom, $P = 0.06$).

Carriers could be divided into two categories occasional carriers (19 out of 54 comprising about one third) and chronic carriers (35 out of 54) In the present study we define a chronic carrier as a subject not showing more than three successive negative swabbings. In fact we can evaluate the shortcomings of the research method by admitting that between the two farthest swabbings positive for the same type the subject normally remains a carrier of the same microorganism Moreover all of the intermediate swabbings should be positive for this type if the technique was completely effective Applying this reasoning to our results we find that this is not so in 20 % of our cases

The number of different types found in the same subject in the course of the study was extremely variable-

Cariers of	Number
1 single type	30
2 different types	11
3 different types	4
4 different types	5
5 different types	3
6 different types	1
	54

Attack by several microorganisms is therefore not rare since nearly a quarter of the children that we have examined harbour three or more different types. The technique that we have used does not allow us to prove that different microorganisms can live simultaneously in the same host but one can reasonably conclude this from the observation that in several carriers of this species the same type can reappear at various intervals.

The most frequently encountered types are 18 3 1* 28 and 32. We also occasionally found types 1 2, 4 5 6 9 11 13, 19 31 and 32.

Correlative serological and clinical study

As we have already emphasized the interpretation of purely bacteriological results *provides scarcely any new information.* From the group studied, we have been able to extract a group of 68 children on whom the clinical and bacteriological study is complete which therefore allows a more precise interpretation. This selection, which results from purely contingent factors—length of stay difficulty of obtaining blood samples etc.—seems to have made *little* modification to our initial sample at least as far as the bacteriological results are concerned, as illustrated in Table 3

Concerning these different categories there are statistically no significant differences between the two groups.

Table 4 summarizes the results of this study

I *Non-carriers* (32 cases, 47 %)

A. Only ten of these 32 subjects were normal" from the serological point of view We can consider them as children unharmed from all contact with streptococcus A during the whole of their stay and the few preceding months.

B (1) Four children showed an elevation of the ASO titre One of them had a recent history of rheumatic fever with mitral carditis; another one probably had an acute infection just before admittance

TABLE 3

	Total group No.	Total group %		Limited group No.	Limited group %	
Total	114	100		68	100	
Non-carriers	60	52		32	47	
Carriers	54	48		36	53	
Carriers, one single type	30	54	of carriers	20	55	of carriers
Carriers, several types	4	46		16	45	

to the hospital. Two others showed an oscillating titre between 200 and 400 units which we were unable to account for not withstanding careful research.

Two girls with normally low serological results presented a high single rise of AH titre. These results cannot be ttributed to a technical error but their significance remains unexplained.

Finally one child showed a marked rise of ADPN titre only over a period of 5 months (128 to 700 units).

(2) Among children who had a concomitant elevation of all the serological reactions only 4 showed definable clinical symptoms (recent rheumatic fever acute pharyngitis, enormous permanent cryptic tonsils with several inflammatory phases) Of the 11 remaining we have no proof of definite streptococcal infection. The raised level of antibodies is concerned most often with the association of ASO with AH (10 cases) though in half of these cases this rise seems more related to other anti body increases. In the case of 7 of the children we observed febrile periods with bronchial infection of the cavum or the sinuses requiring local or general treatment with antibiotics, but we were unable to isolate the group A streptococcus.

In conclusion, it can be seen that this group of "non-carriers is not at all homogeneous with respect to the streptococcal attack. Only fifteen per cent of the

TABLE 4 *Correlative biological and clinical study*

Total of 68 hildren.

I. *Non-carriers*			
A: with low antibody titre		10 (15 %)	32 (47 %)
B: with discordant serology			
(1) one single reaction	7 (10 %)		
(2) two or more reactions with definite antecedents	4 (6 %)	22 (32 %)	
no definite antecedents	11 (17 %)		
II. *Occasional carriers*			8 (11 %)
III. *Chronic carriers*			
A: same type		15 (22 %)	28 (42 %)
B: different types			
(1) with infection by the new type	5 (7.5 %)		
(2) not affected by the new type	3 (5 %)	13 (20 %)	
(3) complex cases	5 (7.5 %)		

total number appear to be finally un affected both bacteriologically and serologically If an original streptococcal infection i debatable in 7 children showing an elevation of a single antibody titre (10 of the total) it is more difficult to dismiss this in the 11 other cases (16 %) where at least two and often several anti bodies are permanently raised

II Occasional carriers (8 cases 11 %)

Eight children belong to this group but in 5 of them there was an interruption in the investigation soon after acquiring the microorganism by external factors (holi days discharge from the hospital etc.) which prevented any analysis

It seems therefore in our group of subjects at least, that the group of occasional carriers are largely artefact due to external circumstances treatment interruptions, etc so that we are not able to draw any conclusions from the restricted group of the 3 true occasional carriers who remain.

III *Chronic carriers* (28–42 %)

Children belonging to this group must be divided into subjects harbouring the same type of group A streptococcus throughout the observation and those exhibiting two or more types

A. *Single type* (15 cases)

(*a*) On six occasions we have been able to witness the acquisition of the microorganism and the development of the infection in a subject until first negative swabbings and low serology are evident, then positive swabbing followed by a detectable elevation of antibodies.

In 3 cases a clear clinical pharyngitis followed the acquisition.

In another case an infectious syndrome with a temperature of 38.5 C occurred after a bronchoscopy the same day as streptococcus A was discovered for the first time in the throat

In one case the clinical examination only disclosed a purulent rhinitis exhibiting no clinical symptoms in spite of a definite serological infection.

(*b*) In a group of 9 other children, also prolonged carriers of a single type we were not able to witness the development of the infection whether the microorganism was already present in the throat at the beginning or whether the serology was already raised, even though delayed positive throat cultures sometimes occurred much later

Generally the serology remains raised as long as the microorganism is present which can last for several months if after several weeks throat cultures are negative then the serological results return slowly to normal It seems that one would be right in a case of this type to conclude that a disappearance of the parasite had occurred

B *Several types* (13 cases)

The biology of these carriers whose behaviour is clearly very special is sometimes complex

(*a*) The most interpretable cases are those in which a child carrier of a given type after having contracted a strain of a new type presents an infection evidenced by an indisputable serological reaction (5 cases)

Only one of these 5 children showed, on this occasion a clinical symptom (pharyngitis).

(*b*) When acquiring the new type three subjects have shown either not any rise

in serology (2 cases) or only a slight change related to a single reaction and during a very short period. It is noticeable that these children present low serological results in spite of a very long coexistence with the microorganism

(c) The remaining five children in our study make up a non-homogeneous group among whom some observations have been interrupted t an important stage of their evolution, thus rendering difficulty in interpretation. This group concerns children carrying successively or simultaneously 2 or more types who retain raised serological antibody titres which remain constant at the appearance of a new type

Discussion

I. *Existence of associated tuberculosis*

The first important question is whether the primary tuberculosis of the children who form the object of our study can influence our results. We discussed this point extensively in another publication [8] and will limit ourselves to the conclusions we reached, which are as follows:

Neither the clinical form of tuberculosis nor the mode of treatment seems to influence the behaviour of these hildren regarding the streptococcal infection.

We were unable to demonstrate the existence of non-specific inhibitors of streptolysin in this group. In any case these aberrant results could concern only 2 non-carriers with high levels of ASO without any previous history relating to a streptococcal infection, that is to say scarcely 3 % of our group.

The average number of carriers is not higher in the group with tuberculous primo-infection than in the normal group;

The limitation which the tuberculous places on our interpretation still stands on two points. First, it may be that this disease is an indirect cause for the relatively high percentage of non-carriers with high serological titres and without previous history of streptococcal infection which our sample presents (17 % at least) As a matter of fact though we have very few comparative studies of the same kind in "normal populations this percentage is somewhat higher than in the studies of Valkenburg *et al.* (9 %) [1] and of Saslaw & Streitfeld (8 6 %) [11], but consistently lower than in that of Packer (25 %) [9] This serological rise may be attributed to extra pharyngeal infective sites, although this hypothesis is very difficult to prove bacteriologically Actually these non-carrier subjects exhibiting a raised level of antibodies probably constitute a *heterogeneous* group which brings together recent infections, subacute infections of extrapharyngeal foci, and perhaps, in the case of tuberculous children, serological revivification by training

Second, the coexistent tuberculosis can facilitate the appearance of antibodies in carriers who otherwise would have low levels of antibodies, that is to say healthy carriers. More studies are needed to appreciate the possible importance of this hypothesis. In the studies mentioned above the percentage of carriers without a rise of antibodies is generally higher than in our study but in those cases the interpretation was based on one single serological technique (ASO) as opposed to the four methods used in our investigation.

With these reservations, the significance

of our results can be examined from the point of view of the method and of the streptococcal infection.

II *Methodology*

The significance of the throat swabbing Unless the streptococcus A is overwhelmingly predominant in the pharynx (which is only encountered in acute pharyngitis) a search for it always seems to be somewhat modified by chance primarily due to the swabbing technique which remains extremely crude

We have found that even using an improved technic one time in five upon swabbing we cannot grow an organism whose presence is highly probable

This presents a double uncertainty the first being that the finding of a negative swabbing does not allow us to assume that the organism is absent In creasing the number of swabs taken in the given time compensates greatly for this shortcoming in the case of chronic carriers, since we generally do recover the organism but we cannot state that there does not exist a group of subjects harbouring the streptococcus for only a very short period of time which our technique would neglect systematically

The second limitation in our interpretation is that we cannot deduce directly from the presence of the organism in the throat (often feebly indicated) that this is the actual focus of infection. Another simultaneous site could well escape our investigations, though being truly the place where the virulence of the microorganism lies for instance when the swabbing is equally positive at the level of another mucous membrane (such as in nasal tissue).

The value of serology It is practically impossible as we have already emphasized to have a precise idea of the biology of the streptococcal infection without a study of the immunological reactions of the carrier The study of the organism, isolated from the culture as far as our knowledge extends today can only lead to an instantaneous static the parasite i fixed at a certain moment of its evolution and placed in a synthetic environment. The dynamic study biological in the real sense of the word, is only approachable by the expedient of investigating the reactions of the host

For several reasons, we are required to carry out a systematic series of several serological tests. One knows in the first place that according to the phase of activity the streptococcus does not produce its diffusible antigens in equal proportions by grouping 3 or 4 reactions we increase the sensitivity of the research, and the confirmation of the infection (in the case of a specific complication) achieves the greatest possible precision. Secondly the error due to the existence of non-specific inhibitors giving excessively false values is therefore reduced, these inhibitors not being all the same for the currently known antigenic enzymes. Finally it is not impossible that the different antibodies in regard to these enzymes have a value and a definitive biological significance this i a possibility whi h we find evident in other work actually in progress.

Moreover we have explained in another paper why it seems to us that properly speaking a 'normal level of anti-streptococcal antibody does not exist [2]. It is undoubtedly not easy to fix the level

from which one could consider that a rise of antibody titre indicates a recent infection. We have fixed this "indicator value at 150 ASO units, 5000 TRU of AH 50 units of ADPN and 6 times the normal level of AK.

III. *Modality of streptococcal infection*

It therefore seems to us to be possible to interpret in some measure, our results in the framework of the streptococcal infection in general.

The method we used, by enlarging the field of our investigations, alters considerably the slant of this interpretation. The clinical method, which defines the illness from the symptoms, uses less systematic criteria allowing chance to take n important part when the illness is not severe, as is most often the case in the subjects we have studied.

Bacteriological diagnosis is here only a rough method and cannot help in defining the infection.

It is therefore the immunology which, in spite of its limitations, gives the most constant criteria of objectivity and which must direct the interpretation. By adopting this point of view it is also true that we are faced with another difficulty If the method which we use is more sensitive it is also less selective and shows on the whole in the formula used, an opposit shortcoming from that of the clinical method.

By shifting the borderline between the subjects who do not have the serological signs and those who do, we regroup in the case of the latter different modes of infection which possess a biological unity but have unequal intensities. On the other hand, with the serological tests used here, we have only explored one aspect of the biology of infection. These tests, the only ones readily practicable on a large scale are only applicable to diffusible enzymes of the streptococcus. Only constituting a partial index of the infection, they do not enable examination of the reactions of the organism in relation to the microbe and in particular of the degree of immunity that it can acquire. We believe that this shortage of facts concerning the serological immunity imposes more limitation on the interpretation of our results than the existence of the associated tuberculosis. These reservations having been made we can draw the following conclusions from our results

Conclusions

1 Children exist who, observed over long periods, remain unharmed from all contact with streptococcus A even when present in a closed environment where the level of infection is high and where the bacteria is very widespread. We have shown statistically in a previous study that this phenomenon is not governed by chance [7] The nature of this immunity remains unknown.

_ Subjects exist who, having acquired the germ and retaining it for a long time, show neither serological nor clinical symptoms, that is to say are true healthy carriers These subjects are very few in our group but their recognition is indisput ble Let us recall, for example that one of our subjects had been a carrier during more than 20 weeks of six different types of streptococcus A with a consistently low serology The question which remains is what proportion these carriers may represent in a normal population. It

has been confirmed that these subjects were not affected by agamma-globulinaemia. On the other hand, they were neither under antibiotic nor hormonal treatment.

3. When the acquisition of a microorganism is accompanied by a serological change, as is most frequently the case, all the intermediate states can be found on the clinical level between the acute illness and the asymptomatic.

The attenuated forms which assume minimal symptomatology where the streptococcus remains dormant seem to be by far the most numerous. This fact is fundamental, *for it implies that the greatest* part of the infections common to streptococcus, which pass unnoticed in practice and escape an active treatment. It does not prove that the attenuated forms can cause complications as frequently as the others, but this persistence of an endemic streptococcus very widespread in childhood (which confirms the more elevated average serology at this age in all the groups of "normal" subjects examined) is an important phenomenon which must be emphasised.

4. The common endemic streptococcus, maintained along with the usual types of streptococcus present in the population under consideration, is sometimes difficult to distinguish from a new strain which can cause a small epidemic movement.

5. The endemic infection often assumes a protracted form and may be prolonged over months. During this period, even in the apparent absence of superinfection, the serology can present fluctuations which may express a perpetual change in the state of equilibrium between the parasite and the host, whose susceptibility to the infection is in this case probably as important a factor as the actual virulence of the microbe.

6. Finally, in the case of chronic carriers, their behaviour with respect to streptococcus A seem to be founded on very different biological bases. One may be a carrier of the same strain over a very long period of time, whereas another shows a definite lability contracting successively different strains. The acquisition of a new type may be accompanied, but not always, by signs of infection. In certain subjects several types may coexist.

Between the subject constantly unharmed *from all contact with streptococcus* A and the one who becomes a victim of a serious complication from a streptococcal infection (for example rheumatic fever) all the various degrees now begin to become clear, as well as certain of the processes which connect them. But the mechanisms that determine the individual behaviour remain unknown. Several ways may be tried to clarify this, but on the clinical biological plane it seems to us now that no actual progress can be achieved without a suitable appraisal of antistreptococcal immunity.

Summary

A longitudinal study has been conducted on 114 children in a hospital for primary tuberculosis in order to determine the incidence of Group A latent streptococcal infection. The subjects were regularly examined for the presence of Group A streptococcus in the throat, the level of streptococcal antibodies in the sera, a daily methodical investigation was also performed in the search of any clinical symptoms of infection.

The analysis of the modes of infection shows that several degrees exist between the subject remaining free of any contact with group A streptococcus and that one who presents a clinically acute infection, some children are truly healthy carriers, but more frequently the acquisition of the germ is followed by a rise of serological reactions. This infection may be clinically latent, or may show clinical symptoms of disease.

Children classified as chronic carriers may harbour a single type of streptococcus or two or more types which may or may not be present concurrently within the carrier

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References

1 Ardelean L., Baldovin Agapi, C., Ionescu, C., Petrescu, A. M., Dumitrescu, V., Gavrilescu, O Grigoriu T Pteancu, A., Klein R., Wisoru F and Vasiliu E.: Observations sur les porteurs de streptocoques hemolytiques dans des collectivités d enfants à caractéristiques épidémiologiques variées. *Arch Roum Path Exp*, *19* 527 1960.
2. Caravano, R.: Le dosage de l'antistreptolysine O Bases de son interprétation pratique *Sem Hop*, *37* 2766, 1961
3. Cornfeld D. and Hubbard J P Four years study on occurrence of beta-hemolytic streptococci in 64 school hildren. *New Engl J Med*, *264* 211 1961
4. Gordis, W R. O Valkenburg, H. A., Bots, A. W and Lorber, J C.: Attack rate of streptococcal pharyngitis, rheumatic fever and glomerulonephritis in a general population. I: A controlled pilot study of streptococcal pharyngitis in one village. *New Engl J Med*, *268* 667 1963.
5. Kirschner, L. and Gallagher, D J A. Studies on hemolytic streptococci I. Factors influencing the carrier rate of the group A hemolytic streptococcus. *New Zeal Med J* *48*, 10, 1949
6. Mozziconacci, P., Gerbeaux, Cl., Caravano, R., Gerbeaux, S., Labonne, J Rahman S., Rabotnska, F Orsaud E. and Virolleau P A study of group A hemolytic streptococcus carriers among school children. I Material, methods and results of the studies. *Acta Paediat Scand*, *49* 711, 1960.
7 — II: Significance of the findings. Ibid. *50* 33, 1961.
8. Mozziconacci, P Gerbeaux, J., Baculard A., Dovnadieu M. and Caravano, R.: Latent streptococcal infection and carrier state during primary tuberculosis in children. T be published in *Amer Rev Resp Dis*, 1965.
9. P cker, H., Arnoult M. B and Strunt, D H.: A study of streptococcal infections in relation t antistreptolysin O titer in orphanage children. *J Pediat*, *48* 845, 1956.
10. Quinn R. W and Martin M P.: Natural occurrence of hemolytic streptococci in school children five year study *Amer J Hyg* *73*. 193, 1961
11 Saslaw M. S. and Streitfeld, M.: Group A beta-hemolytic streptococci in relation to rheumatic fever *Amer J Dis Child*, *92* 550 1956.
12. Valkenburg H A., Gordis, W R. O Bots, A. W Haverkorn van Rijewijk, M., Moor C. E. and Lorber, J C.: The relationship between a positive throat swab for streptococcus pyogenes and antibody response in an open community *Symposium on Streptococcus pyogenes* Jena, October 1963.
13. World Health Organization: Epidemiological and vital statistics report: 15.1 1962.
14. Zanen H. C., Garon, S. and Van Toorn M. J Continuous study of hemolytic streptococci in throats of normal children, adults and aged men. *Amer J Hyg* *69* 265, 1959

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An Abnormality of the Thrombin Fibrinogen Reaction in the Newborn

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It is well known that neonatal plasma takes longer to clot on the addition of thrombin solution of standard strength than does adult plasma. In their textbook Biggs and Macfarlane [3] give figures of 16 to 60 seconds compared to 1° to °1.5 seconds for adult plasma. The investigation described in this paper was prompted by the observation of a grossly prolonged thrombin clotting time during a study of neonatal coagulation. The plasma thrombin clotting times of 43 consecutive samples of cord blood were therefore determined and in three cases the time was grossly prolonged. The defect was corrected by toluidine blue or protamine sulphate None of the infants was bleeding but a similar abnormality has been described in mothers in association with haemorrhagic complications of labour [° 4].

Methods

Umbilical venous blood was collected before delivery of the placenta by venepuncture using a disposable plastic syringe Nine parts of blood were added to one part of 3.8% tri-sodium citrat and well mixed. The plasma was separated by centrifugation at 3000 revs for ten minutes. Thrombin times were performed either immediat ly or after storage for a short time at −20°C

Thrombin clotting times (T C T)

0 1 ml of plasma was mixed with 0 1 ml 0.85 % saline and the clotting time t 37 C determined on the addition of 0 1 ml of a solution of bovine thrombin (Park Davis). The thrombin was dil ted with saline so that the clotting time of normal plasma under the same condition was 15 seconds. Where the control time is different this is stated.

Calcium thrombin clotting times

These were performed as above except that the thrombin was prepared by dil tion in M/40 calcium chloride

In some experiments 0 1 ml of protamine sulphat (100 mg/100 ml) or of toluidine blue (25 mg/100 ml) was substituted for the saline

Plasma fibrinogen

This was measured by the method of Varley [1] modified as follows: plasma 0.. ml was added to 0.5 ml of normal saline and clotted t 37°C with 0 1 ml of bovine thrombin (.5 units/ml) The fibrin was subsequently harvested and estimated as tyrosine as described by Varley In some experiments 0.1 ml of protamine (100 mg/100 ml) was incl ded with the plasma. This addition did not affect the f brinogen determination of three samples of normal adult plasma.

Results

In 40 cases (Table 1) the thrombin time was between °1 and 35 seconds. Included

Table 1 *Thrombin clotting times in 43 consecutive cord bloods* (*in seconds*)

No.	Control	Test	No.	Control	Test	Mother
1	15	21	24	15	35	13
2	15	22	25	15	26	17
3	15	Over 180	26	15	22	15
4	16	27	27	15	21	14
5	15	25	28	15	27	
6	15	30	29	15	22	
7	15	27	30	15	21	
8	15	Over 180	31	15	23	14
9	16	27	32	15	25	16
10	15	25	33	16	35	14
11	16	25	34	15	25	
12	15	24	35	15	30	15
13	15	26	36	16	22	17
14	15	33	37	16	30	
15	16	26	38	15	30	
16	15	23	39	16	30	16
17	15	22	40	15	25	
18	15	Over 180	41	15	25	
19	15	28	42	15	21	
20	16	22	43	16	30	
21	15	23				
22	15	22				
23	15	27				

are cases of Caesarian section, breech delivery toxaemia, meconium stained membranes and liquor and two or three cases where intravenous ergometrine was given before the blood was collected. In 10 cases the blood of the mothers was also tested and the T C.T ranged from 13 to 17 seconds. In one case the mother had a slight ante partum haemorrhage but neither she nor the baby showed unusually prolonged thrombin times. In three cases (Table 2) the thrombin time was over 180 seconds. These three are described in detail below

Case 3

This child was the first born of 21 year old blood group O Rh positive mother who had an uneventful pregnancy Labour started 16 days past the expected day of delivery and lasted 18 hours. 0 5 mg intramuscular ergometrine was given with the birth f the anterior shoulder The mother lost an estimated 15 ozs of blood before the placenta was delivered and 20 ozs afterwards. She was transfused with two pints f blood. The placenta was complete and the membranes incomplete; the whole of the maternal surface was very gritty The foetal surface and membranes were meconium stained.

The child, a girl, weighed 3525 g At birth she was rated Apgar 5 but responded to xygen by face mask and to suction. She was thought to show the clinical signs of placental insufficiency On the third day she showed cerebral irrit tion but on the seventh day a lumbar puncture showed clear cerebrospinal fluid. There is still some suspicion of cerebral damage.

Case 8

This child, a boy was the first born of a 22 year-old mother blood group B Rh positive The pregnancy was uneventful. Labour which lasted 20 hours began three weeks before term. During labour there was a small bright red loss of blood. 0.5 mg of ergometrine was given with the birth of the

TABLE 2 *Summary of investigations in three cases with abnormally prolonged thrombin clotting times*

Case No.		3	8	18
Thrombin clotting time (secs)	Control Test	15 Over 180	18 Over 180	15 Over 180
			With toluidine blue	With protamine
Corrected thrombin lotting time (secs)	Control Test		24 42	5 7
F brinogen (with protamine)			150 mg/100 ml	129 mg/100 ml
Calcium thrombin clotting time (secs)	Control Test			16 1*0

anterior shoulder The mother lost 6 ozs after the birth of the child The placenta showed a small area of infarction at the edge of the foetal surface Retroplacental clot of approximately 2 ozs was noted at the edge of the placenta

The child a boy weighed 1750 g and progressed normally

Case 18

This child a girl was the first born to a 25-year-old mother blood group O Rh positive At 3 weeks the mother had two small vaginal bleed of uncertain origin. There was no evidence of toxaemia. Labour began 6 days before the expected dat and lasted seven hours. 0.5 mg of intramuscular ergometrine was given with the birth of the anterior shoulder The placenta was healthy except for an infarct at the edge The child weighed 3450 g and was linically normal.

The results of coagulation studies per formed on the cord blood of these infants are shown in Table These investigations were sometimes incomplete due to dif ficulty in obtaining sufficiently large samples of cord blood. The thrombin clotting time was grossly prolonged in all three samples. In case 18 calcium accelerated the thrombin clotting time but it still remained very abnormal. Calcium thrombin clotting times of the other two cases were not determined The prolonged thrombin clotting times of cases eight and eighteen were corrected by the addition of toluidine blue and protamine respectively Fibrinogen determination in case 8 was unsuccessful until protamine was added as the plasma would not clot. With protamine added a fibrinogen level of 150 mg was detected in the plasma of this case and of 1*0 mg/100 ml in the plasma of case 18

In summary therefore the plasma of all three cases showed grossly prolonged thrombin clotting times. The level of plasma fibrinogen was moderately reduced in the two cases where this estimation was performed, but not sufficiently to account for the prolonged thrombin clotting time In any event this was corrected by toluidine blue or protamine

Discussion

These observations are important for at least two reasons. In the first place we have found a few cases in the literature where haemorrhage is described in the neonate in association with apparent hypofibrinogenaemia [6 9 11]. In one case fibrinogen was used therapeutically

[11]. Since none of these quote the method of assay nor a thrombin clotting time with protamine correction, it is impossible to be sure if the fibrinogen lack was real or apparent.

Secondly the prolonged thrombin clotting time might contribute to bleeding itself. Since none of our children bled this is likely to be infrequent. Nevertheless we feel that it might be significant in an occasional case. This view is reinforced by the finding of a similar anomaly in maternal blood in association with haemorrhage [2, 4]. The case of Baker & Jacob [2] is of added interest because the child's blood also did not clot. But since it was a fresh stillbirth this must be accepted with caution.

The observation of a prolonged thrombin clotting time in both maternal and foetal blood may indicate that its pathogenesis is related to some placental event. It may be an exaggeration of the mechanism that produces the normal slightly prolonged thrombin clotting time of the neonate. This latter is probably of little clinical significance. Aballi *et al.* [1] have ascribed it to heparin because it is reversed by protamine sulphate and toluidine blue but these would not appear to be adequate grounds. The similar defect noted in the plasma of women during labour was not due to heparin [4]. Prolongation of the thrombin clotting time occurs in states of fibrinolysis [8], and plasminogen activator activity is frequent in the blood of the newborn, [5, 7]. However in fibrinolytic states the thrombin clotting time is not characteristically altered by protamine and we know of no study correlating fibrinolysis and haemorrhage in the newborn. There is in fact insufficient evidence to implicate excessive fibrinolysis as a cause of the prolonged thrombin clotting time observed in neonatal blood.

Gross prolongation of the thrombin clotting time has not been noted in recent reviews of neonatal blood coagulation [1, 10] but we believe that it should be recognised. If it contributes to haemorrhage then it is potentially treatable. If it does not, then a neonate should not be given fibrinogen with possible risk of hepatitis. An association between abnormal thrombin clotting time and excessive bleeding has been shown in adults but further experience is necessary to show if such a relationship exists during the neonatal period. Where an infant has evidence of abnormal bleeding on the first day of life it would seem reasonable to perform a thrombin clotting time before assessing the results of plasma fibrinogen assays and other tests of coagulation function.

Summary

Thrombin clotting times were performed on 43 umbilical cord blood samples. In 3 cases these were grossly prolonged. The importance of this observation is discussed.

Acknowledgement

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References

1 Aballí A. J. and Delamerens, S.: *Coagulation Changes in the Neonatal Period and Early Infancy*. Pediatric Clinics of North America. W. B. Saunders Co., Philadelphia and London. 9/3, 785, 1962.
2. Barry, S. J. and Jacob E.: A haemorrhagic disorder in pregnancy due to an "anticoagulant" preventing the conversion of fibrinogen to fibrin. *J Clin Path 13* 214, 1960
3. Biggs R. and Macfarlane, R. G. *Human Blood Coagulation and Its Disorders* Blackwell Sci. Publ., Oxford 196_, 3rd ed.
4 Bloom A. L. and Campbell, N. The defibrination syndrome with defective thrombin fibrinogen reaction reversible by protamine. *J Clin Path* (in press).
5. Cope, I. and Simmons, E.: The fibrinolytic activity of umbilical cord blood. *J Obstet Gynaec Brit Comm, 65* 414, 19_8
6. De Souza, C. S., Ferreira, J. C., Gomes, A. F. and Estrela A.: La fibrinogénémie du nouveau-né. *Arch Franc Pediat 10* 474 1953
7 Ekelund, L. and Kauer, L.: Fibrinolytic activity in the plasma of newborn infants. *Acta Paediat Scand, 53*, 329 1964.
8. Fluth, P. T.: Haemorrhage and fibrinolysis. *Proc Roy Soc Med, 57* 603, 1964.
9 Hartman J. R., Howell, D. A. and Diamond L. K.: Disorders of blood coagulation during the first week of life. *Amer J Dis Child 90* 594 1955.
10. McElfresh A. E.: Coagulation during the neonatal period. *Amer J Med Sci, 242* 771, 1961
11 Renaerts Bronckers, E. E. and Dekoninck, M. J.: Acute fibrinolysis in newborn infant. *Lancet I* 98_, 1964
12. Varley H. *Practical Clinical Biochemistry*. Heineman, London 1960 2nd ed.

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Effect of Prolonged Fasting and Ketogenic Diet on Levels of Blood Lipids and Ketones in Obese Children

by BENGT E. H. PERSSON and GÖRAN C. G STERKY

Adipose tissue is metabolically highly active and plays a central role in the regulation of plasma free fatty acids (FFA). The main treatment of obesity is caloric restriction. When carbohydrate metabolism is depressed, the oxidation of FFA is increased, leading to an overproduction of ketone bodies. During recent years a great number of metabolic differences between obese and non-obese adults have been demonstrated [14]. Among the abnormal findings are elevated FFA levels after overnight fasting If, however the fasting period is prolonged, the obese display an insignificant FFA increment in contrast to the considerable rise found in normal individuals. Kekwick *et al.* have demonstrated that obese people on a subcaloric high fat diet develop ketosis to a lesser degree than normal people [10].

It is a well known fact that children are more prone to develop ketosis than grown ups. There are, however very few observations on fatty acid metabolism in obese children and adolescents. The aim of the present study was to investigate the effect A) of prolonged fasting and B) of a subcaloric high fat diet on the levels of blood lipids and ketones in growing obese subjects.

Material and Methods

The study was made on 31 obese subjects —20 girls and 11 boys, of ages ranging from $7\frac{1}{12}$ to $16\frac{11}{12}$—without known endocrine disorders. The height–weight tables of Broman *et al.* [3] were used as a standard, and the level of overweight was calculated and expressed in standard deviations (sigma). Weight at birth and height and weight at the age of 7 were also recorded. Certain relevant data concerning the obese subjects are given in Table 1. All subjects were studied while in hospital, to which they had been admitted for obesity

The control subjects, aged 15–18, included 4 girls and 6 boys, of normal height and weight. They were all pupils at boarding school, and the studies were performed there.

Chemical methods Blood glucose was determined according to the Ek–Hultman method [10]. The plasma FFA were analysed by the method of Dole as modified by Trout [31]. Blood ketones were determined as total acetone according to the method of Greenberg and Lester in the modification of Werk *et al.* [33], and plasma glycerol by the enzymatic method described by Wieland [34]. Other serum lipids were determined as follows: total cholesterol according to Cramér–Isaksson [6], phospholipids according to Svanborg–Svennerholm [30] and glyceride-glycerol according to Carlson–Wadström [6]. All analyses were performed in duplicate. The analytical errors for the different methods were: cholesterol 1 1 phospholipids

TABLE 1. *Clinical data in 31 obese subjects: 20 girls and 11 boys*

Case no.	Sex	Birth-weight, g	Age yrs./mo.	Weight kg	Height cm	Overweight sigma	Overweight at 7 yrs, sigma	Type of test
1	F	4340	11/ 2	49.6	142	4.0	3.6	B + A
[illegible]	F	2160	15/ 1	79.2	160	4.3	[illegible]	B
3	M	[illegible]	11/	67.8	[illegible]	6.2	3.7	B
4	F	3200	15/ 5	82.5	162.5	4.1	3.1	B + A
5	F	—	1 / 6	72.6	155	4.2	—	B + A
6	F	[illegible]900	14/ 9	60.9	163	4.7	1.7	B + A
7	F	3930	9/ 5	48.8	143	3.4	4.7	B + A
8	M	3400	11/ 6	74.7	159	5.6	7.3	B + A
9	F	3000	8/	[illegible]	13[illegible]	3.2	3.5	B + A
10	F	41[illegible]0	14/ 9	79.[illegible]	173	2.6	[illegible]	B + A
11	F	3970	13/ 9	66.5	157	2.7	—	B + A
12	F	4160	15/ 6	69.3	167	4.6	3.9	B + A
13	F	—	15/ 6	67.0	169	4.0	1.3	B + A
14	M	3350	13/	63.4	157	3.9	—	B
15	F	—	15/ 6	91.[illegible]	17[illegible].5	4.4	—	B
16	F	4000	14/ 3	79.8	167	3.[illegible]	4.9	B
17	F	3300	13/ 8	84.3	171	3.6	−0.4	B
18	M	3270	16/10	85.4	165	5.6	[illegible]	B + A
19	F	—	14/ 9	84.0	164	4.2	—	B
[illegible]0	M	3100	13/ 5	[illegible]	160	[illegible]	[illegible]	B + A
21	F	5150	8/ 4	54.7	149	2.7	4.1	A
[illegible]	F	[illegible]0	9/10	57.6	141	6.4	7.5	A
23	F	3200	9/ 2	37.4	1[illegible]4.5	6.1	3.5	A
24	F	3700	12/11	79.7	167	3.[illegible]	—	A
[illegible]5	M	4040	12/	60.4	154	3.[illegible]	—	A
26	M	4420	13/ 4	77.8	157	6.4	6.6	A
[illegible]7	M	3900	7/ 7	35.8	130	4.5	5.9	A
[illegible]	M	3360	9/ 2	43.7	137	5.1	3.9	A
[illegible]	M	35[illegible]0	14/10	83.3	171	4.1	3.3	A
30	F	41[illegible]	13/ 5	90.0	175	4.0	1.2	A
31	M	37[illegible]0	7/	40.0	140	6.9	5.9	A

[illegible]9 glyceride-glycerol .8 glycerol 4.4 ketone bodies 6.4 and FFA 4.3 [illegible].

Experimental procedure. Study A was a prolonged fasting of 23 hrs, timed from the last meal at 5 p.m. of the day before the study. While fasting, water was permitted ad libitum, and the subject remained ambulatory though activity was limited.

Venous blood samples were collected after 15, 19 and 23 hrs of fasting. All blood specimens were analysed for blood glucose, ketones, plasma FFA and glycerol. In a few obese cases and in all control cases the serum lipids, cholesterol, phospholipid and glycerides were also determined.

In study B the subjects were given a formula diet for seven days, composed of skimmed-milk powder, calcium caseinate, coconut oil, peanut oil, and cocoa flavouring. The ingredients were mixed with water in blendor giving a calorie value of 49 cal/100 ml, of which 80% was supplied in the form of fat (8.3 % in the form of polyunsaturated f t), 15% of protein and 5% of carbohydrate. (The formula was kindly prepared by Semper AB.)

Using the height–weight tables of Broman *et al.* the mean weight was determined for each of the obese subjects. The amount of calories given was then calculated from the mean weight using the following values:

Age in years	Boys (cal/kg)	Girls (cal/kg)
7 1[illegible]	25	20
13–14	18	16
15–[illegible]0	16	14

TABLE 1 *Clinical data in 31 obese subjects, 20 girls and 11 boys.*

Case no.	Sex	Birth-weight, g	Age yrs./mo.	Weight kg	Height cm	Overweight sigma	Overweight at 7 yrs, sigma	Type of test
1	F	4240	11/	48.6	142	4.0	2.8	B+A
2	F	2150	18/ 1	79.2	160	4.3	2.6	B
3	M	2406	11/	67.8	152	6.2	3.7	B
4	F	3200	15/ 5	82.5	16..5	4.1	3.1	B+A
5	F	—	1 / 6	72.6	163	4.3	—	B+A
6	F	2900	14/ 9	64.9	163	4 7	1 7	B+A
7	F	3930	9/ 5	48.8	143	3.4	4.7	B+A
8	M	3600	11/ 8	76.7	159	5.6	7 3	B+A
9	F	3600	8/	35.9	133	3.2	3.8	B+A
10	F	4120	14/ 9	79.2	173	2.6	..8	B+A
11	F	3970	13/ 9	66.5	157	2.7	—	B+A
12	F	4180	15/ 6	69.3	167	4.6	3.9	B+A
13	F	—	15/ 6	87 0	168	4 0	1.3	B+A
14	M	33..0	13/	63.4	157	3.9	—	B
15	F	—	15/ 6	91.2	172.5	4.4	—	B
16	F	4000	14/ 3	79.8	167	3.3	4.9	B
17	F	3300	13/ 8	85.3	171	3.6	−0.4	B
18	M	3270	16/10	85 4	165	5.5	2.2	B+A
19	F	—	14/ 9	84.0	164	4.3	—	B
20	M	3100	13/ 3	62.8	160	..9	..9	B+A
21	F	5150	6/ 4	34 7	149	2.7	4.1	A
22	F	2850	9/10	57 6	141	6.4	7.8	A
23	F	3200	9/ 3	37 4	1 4.5	6.1	3.6	A
24	F	3700	12/11	70.7	167	3.2	—	A
25	M	4040	12/	58.4	154	3.7	—	A
26	M	4420	13/ 4	77.6	157	6.4	6.6	A
27	M	3900	7/ 7	33.8	130	4.5	5.9	A
28	M	3..50	9/ 2	43.7	137	5.1	3.9	A
29	M	3...0	14/10	83.5	171	4.1	3.3	A
30	F	4160	13/ 5	90.0	175	4.0	1..	A
31	M	3720	7/ 7	60.0	140	8.9	8.9	A

2.9 glyceride-glycerol 2.8, glycerol 4 4, ketone bodies 6.4 and FFA 4.3 ..

Experimental procedure Study A was a prolonged fasting of 23 hrs, timed from the last meal at 5 p.m. of the day before the study While fasting w ter was permitted ad libitum, and the subjects remained am bulatory though activity was limited.

Venous blood samples were collected after 15, 19 and .3 hrs of fasting All blood specimens were analysed for blood glucose, ketones, plasma FFA and glycerol. In a few obese cases and in all control cases the serum lipids, cholesterol, phospholipids and glycerides were also determined.

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Using the height–weight tables of Broman et al. the mean weight was determined for each of the obese subjects. The amount of calories gi en was then calculated from the mean weight using the following values

Age in years	Boy (cal/kg)	Girls (cal/kg)
7 12	..3	20
13–14	18	16
15–20	16	14

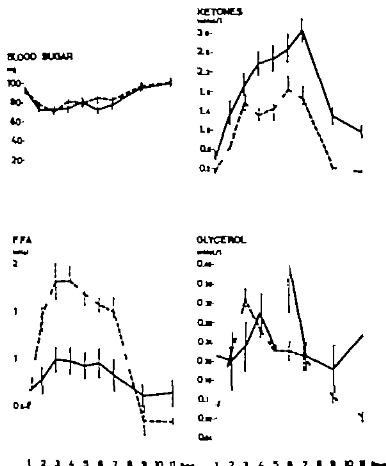


Fig. 2. Mean values ± errors of the mean for blood sugar, FFA, ketones and glycerol during ketogenic diet (Test B). ——— obese; - - - - controls.

Table 4. FFA and ketones (mean values) in test B

Comparison between controls–obese, and subgroups of obese subjects classified according to overweight level at different ages. Δ % stands for peak value minus fasting value in per cent of the fasting value.

	No. of cases	FFA mMol/l	Ketones mMol/l	Δ% FFA	Δ% Ketones
Controls	10	0.61 } –	0.16 }	310 }	1410 } –
Obese Total	20	0.68	0.48	50	899
> 4.0 sigma at the test	12	0.76	0.54	63	807
2–3.9 sigma at the test	8	0.50	0.39	41	1029
> 3.0 sigma at age 7	6	0.92	0.75	23	596
– 0.4–2.9 sigma at age 7	7	0.63	0.28	63	1308

$p < 0.1$. – $p < 0.01$. $p < 0.001$.

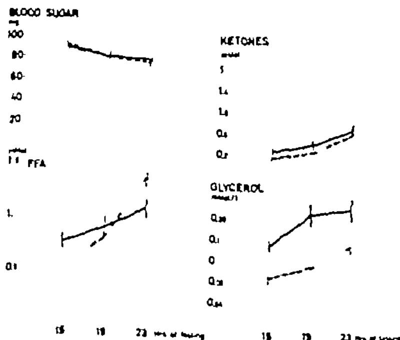


Fig. 1 Mean values ± errors of the mean for blood sugar FFA, ketones and glycerol during prolonged fasting (Test A). ——— obese; - - - - controls.

obtained for the glycerol values. The mean response of blood sugar FFA ketone and glycerol values during prolonged fasting are illustrated in Fig. 1 The statistical treatment of some of these data are given in Table 3. There is a significant correlation between FFA and glycerol after 23 hrs of fasting in the control group. No such correlation is found in the obese group. There is no correlation in either group between blood sugar and FFA or between ketones and FFA.

Table 3 Results of t-test between different hours of fasting (test A) within the obese and control groups.

		Hours of fasting		
		15–19	19–23	15–23
FFA	O	—	—	
	N			
Glycerol	O	—	—	
	N	—		
Ketones	O	—	—	
	N			
Blood sugar	O		—	
	N			

— $p<0.1$. — $p<0.01$. — $p<0.001$

Test B

The mean values for blood sugar FFA, glycerol and ketone bodies before, during and after the ketogenic diet are given in Fig. 2. As in test A, the fasting pre-formula lipid values are higher among the obese subjects, and the ketone values probably significantly higher (Table 4).

Three of ten controls were forced to discontinue the experiment after three days on the diet because of nausea, fatigue and extreme hunger. All symptoms disappeared immediately after feeding with carbohydrates. Two of twenty obese sub

a glucose-fatty acid cycle suggested by Randle et al. [28].

An interesting finding is the significantly higher fasting glycerol value in the obese group. Lipolysis of triglycerides stored in the adipose tissue produces FFA and glycerol. Some of the FFA is re-esterified with glycerophosphate in the adipose tissue. It has been shown in vitro that a small amount of the released glycerol can be reutilized in adipose tissue, probably by means of transphosphorylation to glycerophosphate [5, 20]. The physiological importance of this mechanism is unknown. However, since the free glycerol is probably only poorly utilized by adipose tissue owing to lack of glycerokinase [35], the released glycerol could indicate the degree of lipolysis. It is evident that the higher plasma glycerol levels in the obese subjects could be interpreted as indicative of increased lipolysis if we assume that both groups have a similar distribution and outflux of glycerol from the plasma. The plasma glycerol level could also be influenced by glycerol derived from hydrolysis of triglycerides in the muscles, of triglycerides in the blood, or from the cleavage of glycerophosphate resulting from glycolysis. Experimental evidence indicates a very rapid glycerol turnover rate [24]. A sizable fraction of glycerol is transformed to glucose, the rest being directly oxidized in the liver, kidneys or gut mucosa [16, 35]. A higher rate of lipolysis in the obese subjects might therefore indicate that they have an additional endogenous source of carbohydrate energy in comparison to the controls. This might partially explain why the obese subjects could stand a subcaloric ketogenic diet for a longer period without showing symptoms of possible carbohydrate deficiency [..].

Prolonged fasting affects the two groups differently, the FFA rise being less pronounced in the obese subjects, a finding that is in agreement with observations in adults [14, 23]. This contrasts the findings of Heald *et al.*, which did not exhibit a difference in the FFA response to fasting in a group of obese adolescents [18]. However, all of their subjects had an essentially normal glucose tolerance, probably explained by a relatively short duration of obesity. In all the parameters measured in this study, the obese subjects also develop significant changes at rates much slower than in the normal subjects —a fact that in part could be due to their higher "starting values". The obese subjects do not show the significant relationship between FFA and glycerol found in the controls, which also is suggestive of a different metabolism.

The FFA increment is even less pronounced in the obese group when on the ketogenic diet. The individual ketone and FFA response is, however, much more variable among the obese. When the response is expressed in Δ % (peak value—control value, as a percentage of the latter) it can be shown that the deviation from the normal response increases with rising level of overweight. This abnormality is especially evident when the Δ values are related to the level of overweight at age 7. This finding of an altered FFA metabolism during fasting and caloric restriction is in good agreement with our earlier observation of a lower FFA decrement during glucagon-induced hyperglycaemia [25] and with the observation by Heald *et al.* of a diminished FFA response to exogenous

jects had to give up for the same reasons after six days

There was an insignificant fall in weight both among controls and obese subjects.

The control group exhibits a uniform response with a highly significant rise in FFA and glycerol and fall in blood sugar on the third day and a highly significant rise in ketones on day six. The response among the obese is more variable but the mean rise in FFA is probably significant on day three. There is a highly significant rise in ketones and fall in blood sugar on day seven and six respectively. There is no correlation between the peak value for FFA and blood sugar or ketones in either group. The glycerol values in the obese group are few and were not subjected to statistical treatment. The maximal elevation in FFA and ketones during the test is expressed as $\Delta_{\%}$, i.e. the difference between peak and control levels expressed as a percentage of the control value. There is no correlation between Δ FFA or ketones and sex or age.

The mean birth weight among the obese was 3070 g for girls and 3600 g for the boys, slightly higher than in a control group (for references see Börjeson [4]).

The obese group was divided into subgroups according to the overweight level at the time of the test or at the age of seven. Height and weight data for the age of seven were not available for all the subjects, however. Those with the highest level of overweight deviate most from normal subjects in all the variables measured (Table 4).

Glucose tolerance

Intravenous GTT's were performed in 17 obese cases two days before test B. The mean k_G was 1.1 (range 1.6–0.8). In seven subjects with a mean pre-test k_g of 1.1 the IGTT was repeated the morning after the end of test B. The mean k_g was then 0.7.

Discussion

This study has demonstrated that obese children and adolescents, contrary to findings in obese adults, show a rise in blood ketones similar to or even exceeding that in a control group when subjected to prolonged fasting or on a ketogenic diet. On the other hand, the fasting values for all the blood lipids measured are higher in the obese subjects indicating an altered fat metabolism. They also exhibit impaired glucose tolerance, the mean disappearance rate of intravenously injected glucose (k_g) being 1.1 as compared to 1.63 in normal children of the same age [11]. Similar findings have been demonstrated by Vajda et al. [32]. Conditions involving impaired carbohydrate tolerance as seen in states of diabetes, obesity and starvation have been shown to be associated with elevated plasma FFA levels [1, 9, 17]. If the FFA level is artificially increased (lipid infusion [13, 27] or heparin injection after a fatty meal [29]) the glucose tolerance decreases. Carlson & Östman on the other hand have recently demonstrated in two diabetic subjects with initially high FFA levels, that nicotinic acid lowered the FFA and improved the glucose tolerance [7]. Our findings of elevated FFA combined with a low k_G value exaggerated ($k_G = 0.7$) when the FFA level rises under the influence of a subcaloric high fat formula, together with the above observations supports the hypothesis of

a glucose-fatty acid cycle suggested by Randle et al. [28].

An interesting finding is the significantly higher fasting glycerol value in the obese group. Lipolysis of triglycerides stored in the adipose tissue produces FFA and glycerol. Some of the FFA is re-esterified with glycerophosphate in the adipose tissue. It has been shown in vitro that a small amount of the released glycerol can be reutilized in adipose tissue probably by means of transphosphorylation to glycerophosphate [5 20]. The physiological importance of this mechanism is unknown. However since the free glycerol is probably only poorly utilized by adipose tissue owing to lack of glycerokinase [35], the released glycerol could indicate the degree of lipolysis. It is evident that the higher plasma glycerol levels in the obese subjects could be interpreted as indicative of increased lipolysis if we assume that both groups have a similar distribution and outflux of glycerol from the plasma. The plasma glycerol level could also be influenced by glycerol derived from hydrolysis of triglycerides in the muscles, of triglycerides in the blood, or from the cleavage of glycerophosphate resulting from glycolysis. Experimental evidence indicates a very rapid glycerol turnover rate [4]. A sizable fraction of glycerol is transformed to glucose, the rest being directly oxidized in the liver kidneys or gut mucosae [16, 35]. A higher rate of lipolysis in the obese subjects might therefore indicate that they have an additional endogenous source of carbohydrate energy in comparison to the controls. This might partially explain why the obese subjects could stand a subcaloric ketogenic diet for a longer period without showing symptoms of possible carbohydrate deficiency [2]

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insulin [18]. The present data does not, however permit any conclusions concerning the mechanism behind this abnormal metabolism.

Among the various possible aetiological explanations for obesity Mayer has strongly emphasized the genetic factors [22]. His hypothesis is chiefly based on metabolic observations in the hereditary obese hyperglycaemic syndrome in mice, whose metabolic characteristics—abnormal carbohydrate metabolism abnormal fat metabolism and resistance to ketosis—are very similar to the findings in certain adult obese humans, a state referred to as metabolic obesity [14 15 21]. When studying the significance of genetic factors in overweight children, Börjeson concludes that children who show overweight from the age of 7 "constitute positive extremes in a continuous variation which is largely due to multifactorial inheritance" [4]. Seen against this background, it seems justified to interpret the deviations from the normal in our obese subjects as an adaptation to the obese state per se. Apparently the duration of obesity might be a factor that determines the degree of abnormality. It is also possible that our obese subjects will develop a resistance to ketosis with increasing duration of obesity. The length of such an adaptive period could well be influenced by variations in hormonal balance and the more anabolic metabolism during the period of growth. Before drawing any conclusions concerning this hypothesis, it is necessary to know the length of time needed for such an adaptation to take place, and whether the metabolic abnormality could be normalized during weight reduction. In this connection it is also important to consider the typical metabolic state during the neonatal period and infancy as well as during periods of physiological fat spurts in non-obese children [4 12, 20].

Summary

Blood levels of glucose ketones and various lipids have been studied in 31 obese and 10 normal children during prolonged fasting (23 hrs) and a week on a subcaloric high fat diet.

The obese group has higher fasting values of all components analysed, except blood sugar. A statistically significant difference is obtained for the glycerol and ketone levels. The obese show blood changes later than the normal subjects, have a less pronounced rise in FFA, but a similar rise in blood ketones. The obese subjects had an impaired glucose tolerance that became more pronounced under the influence of the ketogenic diet.

The pattern of response in various blood components among the obese children was best correlated with the time of onset of overweight, i.e. a greater deviation from the controls after a long duration of obesity.

The metabolic abnormalities among overweight children as measured in this study are interpreted as being suggestive of an adaptation to obesity as such duration of overweight playing a central role.

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References

1. BIERMAN, E L., DOLE, V P and ROBERTS, T N An abnormality of non-esterified fatty acid metabolism in diabetes mellitus. *Diabetes, 7* 109, 1958.
2. BLOOM, W L. and AZAR, G. J.: Similarities of carbohydrate deficiency and fasting. *Arch Int Med, 112.* 333, 1963.
3. BROMAN, B., DAHLBERG, G and LICHTENSTEIN, A.: Height and weight during growth. *Acta Paediat Scand, 30*: 1, 1942.
4. BÖRJESON M.: Overweight children. *Acta Paediat, Scand,* Suppl. *132,* 1962.
5. CAHILL, G. F., JR. LEBOEUF B. and RENOLD, A. E.: Factors concerned with the regulation of fatty acid metabolism by adipose tissue. *Am J Clin Nutr 8* 733, 1960.
6. CARLSON L. A. and WADSTRÖM, L.: Determination of glycerides in blood serum. *Clin Chim Acta, 4* 197 1959.
7. CARLSON L. A. and ÖSTMAN, J Inhibition of the mobilization of free fatty acids from adipose tissue in diabetes. II. Effect of nicotinic acid and acetyl salicylate on blood glucose in human diabetes. *Acta Med Scand,* 1965. (In press).
8. CRAMÉR, K. and ISAKSSON B.: An evaluation of the Theorell method for the determination of total serum cholesterol. *Scand J Clin Lab Invest, 11.* 213, 1959.
9. DOLE, V P Relation between non-esterified fatty acids in plasma and metabolism of glucose. *J Clin Invest, 35* 150, 1956.
10. EK, J and HULTMAN E. Determination of glucose and laevulose in body fluids. *Nature, 181* 780, 1958.
11. VON EULER, U and LARSSON Y Glucose tolerance tests in children; a methodological study *J Clin Lab Invest, 14* 62, 1962.
12. VON EULER, U LARSSON Y and PERSSON B.: Glucose tolerance in the neonatal period and during the first six months of life. *Arch Dis Childh, 39*: 393, 1964.
13. FELBER, J P and VANNOTTI, A.: Effects of fat infusion on glucose tolerance and insulin plasma levels. *Med Exp, 10* 153, 1964.
14. GORDON, E. S., GOLDBERG, M. and CHOSY G J A new concept in the treatment of obesity *JAMA, 186* 50, 1963.
15. GORDON, E. S. et al.: Abnormal energy metabolism in obesity *Transactions of the Association of American Physicians, XXV* 118, 1962.
16. HAESSLER, H. A. and ISSELBACHER, K. J The metabolism of glycerol by intestinal mucosa. *Biochim Biophys Acta, 73.* 427 1963.
17. HALES, C. N. and RANDLE, P J Effects of low carbohydrate diet and diabetes mellitus on plasma concentrations of glucose, non-esterified fatty acid, and insulin during oral glucose-tolerance tests. *Lancet* ?90, 1963.
18. HEALD F P., MUELLER, P and DAUGELA, M.: Glucose and free fatty acid metabolism in obese adolescents. *Am J Cl Nutr 14* 258, 1964.
19. KEKWICK, A., PAWAN G L S and CHALMERS, T M.: Resistance to ketosis in obese subjects. *Lancet,* ii. 1157 1959
20. MARGOLIS, S. and VAUGHAN M α-glycerophosphate synthesis and breakdown in homogenates of adipose tissue *J Biol Chem, 237* 44, 1962.
21. MAYER, J *Metabolism of the adipose tissue in the hereditary obese-hyperglycemic syndrome. Fat as tissue.* Ed. by K. Rodahl and B. Issekutz, McGraw Hill Book Co New York, 1964
22. — Genetic factors in obesity *Bull N Y Acad Med, 36* 323, 1960.
23. MOSER, W L and MAKAREM, R.: Changes in glucose tolerance and plasma free fatty acids after fasting in obesity *Diabetes 13.* 286, 1964.
24. NIKKILÄ, E. A. and OJALA, K.: Glycerol as an endogenous precursor of body glucose in the rat. *Ann Med Exp Fenn, 41* 470, 1963.
25. PERSSON B., LARSSON Y and STERKY G Effect of glucagon on plasma level of free fatty acids. *Acta Paediat Scand,* Suppl. *135* 165, 1962.
26. PERSSON B and GENTZ, J The pattern of blood lipids and ketones during the neonatal period, infancy and childhood. (In manuscript.)
27. PERSSON, B STERKY G and THORELL, J Unpublished observations.
28. RANDLE, P J GARLAND, P B., HALES, C. N and NEWSHOLME, E. A. The glucose-fatty acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus *Lancet i.* 785, 1963.
29. SCHALCH, D. S. and KIPNIS, D M. The impairment of carbohydrate tolerance by elevated plasma free fatty acids. *J Clin Invest, 43.* 1283, 1964.
30. SVANBORG, A. and SVENNERHOLM, L Plasma total lipid, cholesterol, triglycerides, phospholipids and free fatty acids in healthy Scandinavian population. *Acta Med Scand, 169* 43, 1961
31. TROUT D ESTES, E. H. and FRIEDBERG, S. J Titration of free fatty acids of plasma. A study of current methods and new modification. *J Lipid Res, 1* 199, 1960.
32. VAJDA, B HEALD F F and MAYER, J Intravenous glucose tolerance in obese adolescents. *Lancet i.* 902, 1964

33. Werk, E. E., Jr., McPherson, H. T., Hamrick, L. W. Jr., Myers, J. D. and Engel, F. L.: Studies on ketone metabolism in man. A method for the quantitative estimation of splanchnic ketone production. *J Clin Invest, 35*: 1256, 1956.

34. Wieland, O.: Eine enzymatische Methode zur Bestimmung von Glycerin. *Biochem Ztschr 329*: 313, 1957.

35. Wieland, O. and Suyter, M.: Glycerokinase. Isolierung und Eigenschaften des Enzyms. *Biochem Ztschr 329*: 320, 1957.

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Childhood Diabetic Neuropathy

A Clinical and Neurophysiological Study

by O EEG-OLOFSSON and I PETERSÉN

Functional disturbances of neurological character in cases of diabetes mellitus have been recognized and studied since the end of the last century Cases described in the literature deal almost solely with adults, i.e. diabetics more than 16 years of age. The reported incidence of neuropathy has varied between 25 and 90 % [4], with symptoms varying in degree of severity from very slight ones to those causing chronic disability Involvement of the central, peripheral and autonomic nervous systems has been observed. Peripheral neuropathy is the most common complaint, with sensory symptoms being more frequent than motor ones, and occurring more commonly in the legs than in the arms. In addition to the clinically manifest form of neuropathy with diabetes there has also been observed a latent, subclinical form which it has been possible to verify by means of electrophysiological methods, such as electromyography and determination of the conduction velocity in peripheral nerves. Up to the present, the majority of investigations carried out have referred to peripheral motor nerve [5 16, 27 31 34, 35 4], but investigations of peripheral sensory nerve have also been reported [5 7 34] Autonomic neuropathy has also been studied comparatively closely [1 30] Involvement of the central nervous system, first and foremost the brain, on the other hand, is less well known, with the exception of functional disturbances secondary to vascular involvement. Solitary electroencephalographic examinations of adult diabetics have been reported [3 10, 20 23 26, 46].

Contrary to the comparatively extensive studies of diabetic neuropathy in adults, diabetic neuropathy in childhood has not received much attention. This may be due to the fact that clinically obvious neurologic disturbances are very rare in children with diabetes, the incidence being about 1.3 % in diabetics below the age of 20 [28, 33 39]. A number of facts, however appear to indicate that a sub-clinical form of neuropathy may not be uncommon in children. Electrophysiological examinations of diabetic children have largely consisted of determination of nerve conduction velocities (NCV) Reports of examinations by means of electromyography (EMG) cannot be traced, and reports of electroencephalographic (EEG) findings in diabetic children are infrequent.

In view of these findings a combined

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investigation involving EEG EMG NCV and clinical analysis was undertaken in a group of diabetic children. A large series of clinically normal children were selected as controls [36].

Material and Methods

This material is composed of 85 children between the ages of 2 and 16 years and comprises the majority of 108 continuously observed diabetics between the ages of and 16 years. All were evaluated at the Gothenburg Children's Hospital and all except one reside within the city limits of Gothenburg. For purposes of this study the children included were randomly selected from the known group of diabetics less than 16 years of age residing in Gothenburg. The average age was 10 years. The duration of diabetes varied from 0 to 14 years, the mean duration being 4.6 years. In 50 cases (59) the onset of clinical diabetes was between the ages of 1 and 9 years, and in 35 cases (41%) between the ages of 9 and 15 years. In no case could any clinical symptoms indicating vascular disease, retinopathy (examined by an ophthalmologist), nephropathy hypertension or neuropathy be found. All except 2 of the 85 children were being treated with insulin. The material was classified into patients who in excellent" "fair or "poor" control (internationally used terminology). In our material 33%, 29%, 38% falling to each group respectively Note was taken of glycosuria, habitual diet and willingness to cooperate the latter two factors being evaluated by the child attending physician. Diabetic coma had, on one occasion or other occurred in 6% of the total cases, whereas the incidence of hypoglycaemic coma in the same group was 21 %. Repeated electrophysiological registrations have been taken on many children, but only the initial registration is included in this material. EEG recordings were taken on all the 85 cases, EMG's in 74 cases, peroneal NCV's in 58 cases, and ulnar NCV's in 6_ cases. The control material is composed of children selected from the patients attending the Clinical Neurophysiological Laboratory of the Sahlgren Hospital, Gothenburg [36]. EEG registrations were done on 410 of the control cases, peroneal NCV's in 135 cases, and ulnar NCV's in 149 cases. EMG studies were done on the same control cases in whom NCV was performed. Both the diabetic and the control material were divided into age groups which, with respect to the EEG material, are 2–3 years, 4–6 years, 7 10 years and 11–15 years, and with respect to the NCV material are 3–7 years and 8–15 years (Table 1). The classification is different because in the first case we were primarily interested in following EEG development serially whereas, in the second case we merely wished to make a comparison with previously published data.

EEG registrations were taken with either a Grass or a Kaiser electroencephalograph. In the majority of cases eight channels were used for EEG recording and two for recording eye movements. The electrodes were disposed according to the 10–20 electrode system, and conventional longitudinal and transverse bipolar leads were employed. The paper speed was 3 cm/sec. EEG recording at rest occupied the initial 30 minutes and was, as a rule followed by hyperventilation (quick, deep breathing for 3 minutes) and photic stimulation, carried out by means of a Kaiser stroboscope Thereafter sleep activation (either spontaneous or if unsuccessful, barbiturate-induced) was done in 170 children in the control group and 34 children in the diabetic group.

EMG registrations were obtained from one of the small hand muscles, M. interosseus dorsalis I as well as from M. tibialis anterior and M extensor digitorum brevis bilaterally A coaxial needle electrode was used in conjunction with a Disa electromyograph.

NCV registrations were carried out according to Hodes, Larrabee & German [24] with slight modifications. The nerve was stimulated at two different points and the corresponding peripheral muscular response was recorded on the electromyograph. Subsequent to amplification, the muscle action potential was photographed. A single

Table 1 *Material.*

EEG + EMG + NCV 66 cases; EEG + EMG 8 cases (4), EE 11 cases (85).

	Diabetics			Controls		
NCV material	*M*	*F*	*Total*	*M*	*F*	*Total*
3– 7 years	8	7	15	29	18	47
8–15	27	24	51	61	51	102
	35	31	66	80	69	149
EMG-material						
2– 3 years	3	3	6			
4– 6	5	5	10	80	69	149
7 10	7	8	15	3–15 years		
11 15	25	18	43			
	40	34	74			
EEG-material						
2– 3 years	4	4	8	18	10	28
4– 6	4	5	9	50	52	102
7 10	13	11	24	51	47	98
11 15	24	20	44	90	92	182
	45	40	85	209	201	410

monophasic square wave with a frequency of 1000 p/sec was used. The right ulnar nerve was stimulated at two points, proximally at the dorsal ulnar epicondyle of the humerus and distally over the ulnar nerve at the wrist. The response was recorded from the hypothenar muscles. The right peroneal nerve was stimulated posterior to the capitulum fibulae proximally and posterior to the malleolus fibulae distally. The response was recorded from M. extensor digitorum brevis.

A 5% significance level has been used throughout in statistical analysis of the data.

Results

NCV

Comparing the diabetic and control materials, the diabetics proved to have lower nerve conduction velocities except in the ulnar nerve for the age group 3–7 years (Table 2, Fig. 1). The lower conduction velocity in the peroneal nerve and the ulnar nerve for the age group 8–15 as compared to the corresponding control group, is significant. Individual nerve conduction velocities were considered pathological only when the value was more than two standard deviations below that for the corresponding control group. By this definition the conduction velocity in the peroneal nerve was found to be pathologically reduced in 5 diabetics, all of whom belonged to the age group 8–15 years, an incidence of 11% for the age group and of 9% if considered in relation to the entire NCV material. The conduction velocity in the ulnar nerve was found to be reduced in 1 diabetic subject belonging to the ge group 8–15 years.

EMG

With respect to the entire diabetic material, only 4 pathological registrations

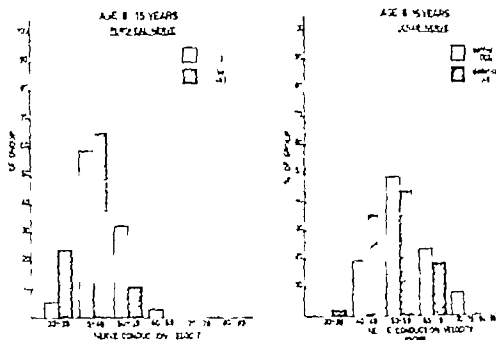


Fig 1 Composition of material according t nerv conduction elocity of age group 8-15 years

were obtained, 1 belonging to the age group 7-10 years and 3 to the age group 11 15 years No fibrillation potentials were noted. In all the diabetic cases a somewhat reduced voluntary activity was recorded from M. extensor digitorum brevis bilaterally and on clinical examination, these muscles were found to be atrophic. In 1 case reduced activity from M tibialis anterior was recorded bilaterally

TABLE Statistical analysis of peroneal and ulnar conduction velocity

Nerve studied	Patient group	No. pts.	Mean velocity (m/sec)	Standard deviation (m/sec)	Standard error of mean (m/sec)	t value
3-7 years						
Peroneal	Controls	43	48	5.6	0.9	1.44
	Diabetics	12	46	4.5	1.3	
Ulnar	Controls	47	53	7 9	1.2	2.74
	Diabetics	14	61	12.5	3.8	
8-15 year						
Peroneal	Controls	92	48	5.9	0.6	4.73*
	Diabetics	46	43	5.5	0 8	
Ulnar	Controls	102	56	8.2	0.8	2.19*
	Diabetics	46	53	6.6	1.0	

Statistically significant t the 5 % level.

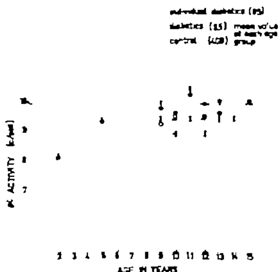


Fig. 2. Alpha activity according to age.

A deviation from normal regarding M. interosseus dorsalis I manus could not be established in any case.

EEG

The basic (alpha) activity appeared at a significantly lower level in the diabetic material than in the control material. This was plainly evident in ages under 9 years, where frequencies below 8.4 c/sec dominated (Fig. 2).

The literature contains a description of a slow posterior rhythm with a frequency of 2.5–4.5 c/sec. It occurs in approximately 0.5 % of adult control material [2, 40]. The existence of this rhythm in healthy children [36] is shown in Fig. 3, which illustrates not only the rhythmic activity but also the age distribution of the first 500 of the selected normal and healthy children. The age groups in this figure have been paired such that 50–100 children comprised each group and each group spans a 3 year period. If sequences of rhythmic waves of minimum 3 seconds are required for approval of a rhythm, the illustrated dashline crests will be obtained. If only one-second sequences are required, then white crests also will be obtained. As will be seen, the relative distribution will be the same whichever method is used. The rhythm appears most frequently in the age group 4–5 years, after which it gradually diminishes.

As far as our diabetic material is concerned, it is not sufficient for division into so many groups. It is evident, however that as regards the existence of rhythms in sequences of minimum 3 seconds, the diabetic material does not differ significantly from the control material.

The effect of hyperventilation was the same in both the diabetic and the control material.

Fourteen and 6 c/sec positive spike-potentials, considered to be diencephalically generated [17], were found in 19 % of the diabetic material, whereas the control

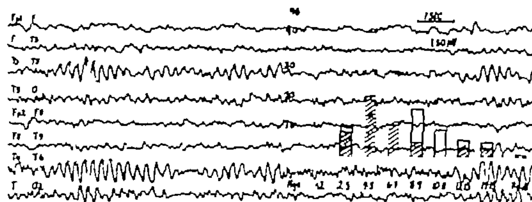


Fig. 3. EEG from one of the controls showing the slow rhythmic activity in posterior leads. Inset, a diagram showing the appearance of this rhythm in different age groups of the control material. Each column represents the rhythm with duration of at least 1 second. [ ] The rhythm with duration of more than 3 seconds.

material showed this activity in only 8 % of the cases. The difference is significant.

A normal EEG was recorded in 87 % of the control material and in 65 % of the diabetic material. Slight to moderate nonspecific abnormalities were found to exist in 10 and 33 % respectively. Paroxysmal activity was recorded in 0 and 8 % respectively. The nonspecific abnormalities consisted primarily of pathologically d theta activity. In 2 diabetic cases the paroxysmal activity consisted of bilaterally synchronous spike activity appearing in sleep; in 1 diabetic case, it appeared in the form of sharp wave-resembling potentials in sleep; in 3 diabetic cases as sporadically appearing spikes or sharp wave potentials on drowsiness and in sleep; and finally in 1 diabetic case as sharp wave potentials subsequent to hyperventilation.

NCV vs Clinical Status

The nerve conduction velocity was related to age at onset of diabetes, duration of disease, control, occurrence of coma, and age and sex of the children. On multiple regression analysis, the nerve conduction velocity proved to be entirely independent of the age of onset of diabetes. On the other hand, a significant negative correlation with duration of diabetes and with age was found, i.e. the conduction velocity in the ulnar nerve and the peroneal nerve diminished with increasing duration of disease and age of the patient (Figs. 4 and 5). It was further noted that, on the average, the cases of "poor" control showed nerve conduction velocities below the mean value. Of the 5 cases with pathologic depression of the conduction velocity in the peroneal nerve, 3 were in "poor" and 2 in "fair" control. In 3 of these cases hypoglycemic coma had occurred at some time and in 1 diabetic coma had occurred. The case of pathologic conduction velocity in the ulnar nerve was in "fair" control but had had both hypoglycemic coma and diabetic coma. The nerve conduction velocity was unaffected by the distribution of the number of boys and girls.

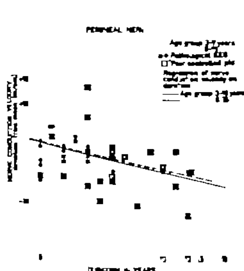


Fig. 4. Peroneal nerve conduction velocity in relation to duration of diabetes. Inserted, regression lines, signs for "poor" control and pathological EEG

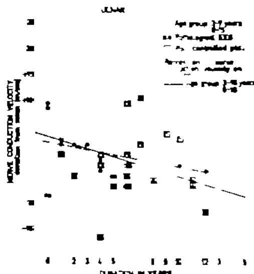


Fig. 5. Ulnar nerve conduction velocity in relation to duration of diabetes. Inserted, regression lines, signs for "poor" control and pathological EEG

EEG vs. Clinical Status

No correlation was found between pathological EEG findings and actual age, age at onset of the disease, duration, control or sex. Of the 19 cases who had had hypoglycemic coma, 11 cases showed a pathologic EEG. This correlation is significant. No significant correlation, on the other hand, was noted between diabetic coma and pathological EEG

The 14 and 6 c/sec activity which occurred significantly more often in the diabetic material than in the control material, was compared with actual age, age at onset of the disease, and with duration of the disease. Due to the fact that all the cases had a duration of less than 6 years, the only significant correlation was in this group. Four of the cases were younger than 11 years, and 6 cases 11 years or older.

EEG vs NCV

Nineteen pathologic EEG s were found in the patients where nerve conduction velocity in both the peroneal and the ulnar nerves had been done With regard to nerve conduction velocities below the mean value, there was an over representation of pathologic EEG s. Of the 5 cases of pathologic conduction velocity in the peroneal nerve 4 cases showed pathological EEG s. The only patient with pathologic conduction velocity in the ulnar nerve had a normal EEG

Alpha activity vs. NCV

In the control material, the conduction velocity in the peroneal and the ulnar nerves remained practically constant with increasing alpha frequency just the same as in the diabetic material as far as the peroneal nerve is concerned. On the other

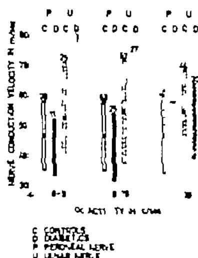


Fig. 6. Nerve conduction velocity in relation to alpha activity. The black squares in the center of the columns represent the mean value. The ends of the columns represent the upper and lower limit, respectively, of twice the standard deviation from the mean value. The numerals on the top of the columns refer to number of cases.

hand, a slowing of the conduction velocity in the ulnar nerve was observed in the diabetic material with increasing alpha frequency (Fig. 6).

Discussion

Determination of the conduction velocity in motor nerves has proved valuable in the study of functional disturbances in peripheral neurologic disease. The method applied was introduced by Helmholtz in 1850 [21] and has been used in many places. During the initial years of life, children have a lower nerve conduction velocity than adults due to the degree of maturity of the nerve. Thomas & Lambert [45] showed that 3–7 weeks prematurely born babies had conduction velocities in the ulnar nerve of 18–22 m/sec, whereas newly born fully developed babies showed values of 21–33 m/sec, i.e. approximately half of the conduction velocity of adults. By three years of age most of the values were at the lower limit of the 95 % confidence interval for the conduction velocity of adults, and by five years of age there was no significant difference to be found. Gamstorp [14] found that the conduction velocity in the peroneal and the ulnar nerves in newborn babies was half the corresponding velocity in adults, and that the conduction velocity in children older than 3 years had reached the same value as for adults. Our control material confirms these observations.

When evaluating NCV, however, it is necessary to take into consideration the existence of possible sources of error. Henriksen [22] found that nerve conduction velocity was reduced at the rate of 2.4 m/sec per degree Centigrade drop in temperature, and Johnson [27] indicated a reduction of 5 % per degree. The temperature in the lower extremities is lower than in the arms, and this partly explains the lower conduction velocity in the peroneal nerve as against the ulnar nerve. As essential sources of error Gassel [16] mentioned abnormal innervation, the measuring of potentials generated by muscles at a distance from the electrode, and spreading of the stimulus to nerves other than the one above which the electrode has been positioned. Differences of 3.5–10 m/sec were recorded. Simpson [41] showed that erroneous measurement of the distance between the two points of stimulation of the skin, as well as erroneous reading of the period of latency on the film, at the most, could produce differences amounting to 11–12 m/sec. Examinations of the same patient at a 4-hour interval could show differences up to 7 m/sec. The

author therefore emphasized that no borderline findings should be accepted as pathological. In our material we have included as pathological nerve conduction velocities only those values which were found to be lower than twice the standard deviation from the mean value of the control material, and in so doing, we consider that we have taken into account the various sources of error referred to above. A whole range of factors having an influence on the nerve conduction velocity has been exhaustively discussed by Škorpil [43].

On statistical analysis of the determinations of nerve conduction velocity a significant increase of the velocity in the ulnar nerve in the age group 3–7 years was verified in the diabetic material. This group totaled 14 children, 3 of whom showed conduction velocities of 80 82 and 87 m/sec respectively The highest rate in the corresponding group of the control material was 79 m/sec. Since nerve conduction velocities higher than twice the standard deviation above the mean value, however have no known pathologic significance these findings have been regarded as of no importance. One child with ccelerated nerve conduction velocity has been re-evaluated and the nerve conduction velocity remained unchanged.

Pathologic conduction velocities in the peroneal and the ulnar nerves were noted in 9 and 2 % respectively of our diabetic material. Gamstorp [15] defines the conduction velocity in the peroneal nerve as pathologic in 1 case out of 45 examinations of 23 children without neuropathy No pathologic conduction velocity occurred in the ulnar nerve The author observed statistically significant lower conduction velocities in the ulnar median, and peroneal nerves of those children between the ages 3–16 years on comparison with a previously published control material [14]. Lawrence & Locke [3_] found pathologic conduction velocities in the peroneal nerve of 4 of 21 children aged 6–15 years with diabetes without clinical neuropathy an incidence of 19 %. Skillman *et al* [42] found pathologic conduction velocities in the peroneal nerve in 17 % and in the ulnar nerve in 10 %, of a mixed group of 103 adults and children with diabetes without clinical neuropathy The youngest patient was 1_ years of age Hoffman [_5], probed the conduction velocity in the ulnar and peroneal nerves on 30 diabetic children of ages ranging from 2–17 years and compared the results with 27 control children of the same ages without showing any statistically significant difference. Moreover data relating to NCV in children with diabetes have been published in connection with examinations of adult diabetics by Mulder *et al* [35] (of 103 patients examined, 4 were between 11 and 19 years) and by Mayer [34] (of 23 patients under 35 years of age the youngest was 10 years old).

The significantly reduced conduction velocity for both the peroneal and the ulnar nerves in the age group 8–15 years, showed a negative correlation in relation to both increasing age and duration of diabetes. Depression of NCV was also obviously related to poor control. The data referring to the relationship between peripheral neuropathy and clinical data available in the literature differ widely [1 31 34, 43, 44]. The most important parameters, however are duration and control.

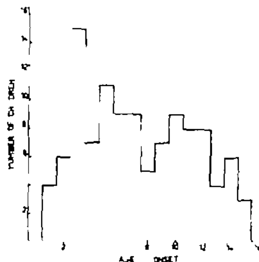


Fig. 7. Composition of all diabetic children of Gothenburg according to age at onset of diabetes. The shaded area represents the 35 diabetic children here reported.

A comparison not previously referred to in the literature between nerve conduction velocity and alpha activity in EEG registrations was undertaken and carried out. Apart from a single exception, the practically constant nerve conduction velocity with increasing alpha frequency confirms the rapidity with which peripheral nervous function develops in relation to the slower development of the central nervous system. The fact that the conduction velocity in the ulnar nerve in the diabetic material shows a diminishing trend, is due to the 3 above-mentioned nerve conduction velocities exceeding 80 m/sec one of which falls within the alpha activity group 8–9 c/sec and two in the group 9–10 c/sec thus causing a rise of the average values of these two groups and resulting in the former showing the greatest effect of the rise.

In the diabetic material, alpha frequency was significantly lower than in the control material in which alpha frequency increases successively with increasing age. In the diabetic material there is no tendency toward an increase in alpha frequency in ages under 9 years, where frequencies below 8.5 c/sec dominate. At 9 years of age, there is a sudden increase of alpha frequency which thereafter runs parallel with alpha activity in the control material. The cause of this sudden change has not been established. It is possible that it may have to do with the existence of two different types of diabetes in childhood. This theory is supported by the fact that in our diabetic material we have been able to trace two age-onset groups—one up to 8 years of age having its peak incidence between 3 and 6 years of age, and another from the age of 9 years upward having its peak incidence between the ages of 10 and 13 years (Fig. 7).

The frequency of pathological EEG registrations in diabetic material consisting solely of adults or adults and children more than 13 years, reported in the literature, varies considerably. Greenblatt *et al.* [20] verified pathologic activity in 28 %, Izzo *et al.* [26] in 61 %, Andersson & Kirstein [3] in 49 % and Herrlin *et al.* [23] in 31 %. In none of these materials has any mention been made of comparative control material, and to a certain extent this may explain the variations. In our child diabetic material, 35 % pathologic EEG registrations were verified, whereas the corresponding frequency of the control material was 13 %. There is no material of diabetic children for comparison. Single cases have been reported by Engel [10] and Wilson [40].

Like other authors, we found a correlation between pathologic EEG registrations

and severe attacks of hypoglycemia. Repeated episodes of this type cause functional and metabolic disturbances in brain cells but are probably of minor importance as causal factors since diabetic patients with no sign of hypoglycemia also have pathologic EEG tracings. Genetic factors are important determinants of pathologic EEG patterns, and it is our opinion that diabetic children with pathologic EEG s as a sign of unstable cerebral, especially cortical, function have a tendency to show severe insulin reactions repeatedly

The over-representation among the diabetic children of 14 and 6 c/sec positive spike activity which we found has not previously been reported. These paroxysmal phenomena are considered to be diencephalically generated [17]. The activity is present in childhood, starting at the age of 4–5 years, and reaches its greatest intensity between 8 and 9 years of age. Thereafter it diminishes. Gibbs & Gibbs [17 ref. in 38] estimated the frequency among non-selected child material as 5 %, whereas Kellaway et al. [29] arrived at a figure of – % in a similar material. In our specially selected control material [36] the activity was found to be 8 %. At this stage it is too early to make any judgment regarding the importance of the high frequency (10 %) found in the diabetic material. The findings, however appear to indicate an increased incidence in children with short duration, and possibly late onset of the disease.

Neither in the matter of EMG registrations is there any child diabetic material for purposes of comparison. In adult patients, Mulder et al [35] traced 10 % pathologic EMG among 52 diabetics who showed no clinical signs of neuropathy whereas Fagerberg et al. [12] found 13 %. Our own lesser figure of 5 % fits very well, considering the relationship between adults and children as regards nerve conduction velocity which, as shown above depends on age and duration of disease

With the object of studying a possible relationship between peripheral neuropathy and capillaropathy skin biopsy from the dorsal aspect of the foot of 28 diabetic children [4] was carried out. Preliminary studies have indicated that capillaropathy is comparatively rare and is difficult to diagnose on light microscopic investigation. There is a clear discrepancy between latent peripheral neuropathy and capillaropathy in this material.

The pathogenesis of diabetic neuropathy is still unexplained. Different hypotheses have been advanced, vascular nutritive factors [11] or metabolic factors [5 6 19] are considered to be the most probable. In favor of a vascular cause of neuropathy in cases of diabetes are the known pathologic changes in kidneys and retina of capillaropathy type; that neuropathy and retinopathy in adult diabetics run parallel, and that neuropathological investigations tend to show changes in vasa nervorum in adult diabetics. Support in favor of the metabolic hypothesis is to be found in Eliasson [9] experiments with alloxan diabetic rats and rats subjected to pancreatectomy In both groups, a reduction of the nerve conduction velocity by 30 % was registered. He showed that the diabetic condition was associated with defective incorporation of acetate and glucose into nerve lipids [8], a fact which may possibly affect the thickness and quality of the myelin sheath. Field & Adams [13], showed regression of similar pathologic changes in

rabbits with administration of endogenous insulin.

In our material there are no signs of retino- or nephropathy diabetic complications generally associated with vascular changes. Neither are there any definite light microscopic findings indicating capillaropathy in the skin. The observed latent peripheral neuropathy is correlated with age duration of diabetes and obviously to poor" diabetic control. These circumstances, together with the fact that the material takes the *form of individuals of* a maximum age of 15 years, appear to indicate that the primary pathogenic cause of diabetic neuropathy is of metabolic character

Summary

Electrophysiologic studies and a comparative analysis of clinical data have been done on 85 children aged .–15 years, with diabetes mellitus but with no clinical signs of neuropathy or known "complications" in any other respect. Electroencephalographic registrations were taken all 85 cases, *electromyography in* 74 cases, and determination of the conduction velocity in peripheral motor nerve in 66 cases. The results were compared with a specially selected control material.

1 A statistically significant reduction of the conduction velocity was noted in the ulnar and peroneal nerves in the diabetic material as compared to the control material for the age group 8–15 years. Pathologic conduction velocity was found in the ulnar nerve in 2% and in the peroneal nerve in 9%. The reduced nerve conduction velocity was related to age duration of diabetes and obviously to "poor' diabetic control.

2. The electromyographic analyses showed a pathologic pattern in 5% of the cases—all from the lower extremities.

3 Pathologic EEG s were noted in 35% of the diabetic material and in 13% of the control material, a difference which is statistically significant. No relation to age age at onset of diabetes, duration of disease or diabetic control could be found. On the other hand, there was a statistically *significant correlation with the frequency* of hypoglycemic coma. The alpha activity was statistically significantly lower in the diabetic material as compared with the control material. This difference was pronounced below 9 years of age Because of this finding, as well as the fact that an age limit of 9 years divides the material into two onset groups, the existence of two different types of childhood diabetes was discussed. In 19% of the diabetic material there were 14 and 6 c/sec positive spike potentials, whereas the control material showed an incidence of only 8 .. This difference is statistically significant. The findings appear to indicate an increased prevalence of this activity in cases of diabetes of short duration, and possibly in cases of *late onset of diabetes in childhood.* Alpha activity was compared with the nerve conduction velocity In both control and diabetic material the nerve conduction velocity remained constant with increasing alpha frequency

4. The pathogenesis of diabetic neuropathy has been discussed. Metabolic factors are considered to constitute the primary cause.

References

1. Aagenaes, Ö.: *Neuromuscular examinations on the lower extremities in young diabetics. With special reference to the autonomic neuropathy.* Thesis, Copenhagen, 1962.
2. Aird, R. B. and Gastaut, Y. Occipital and posterior electroencephalographic rhythms. *Electroenceph Clin Neurophysiol, 11* 637 1959.
3. Andersson B. and Kirstein, L.: Elektro-phalographische Befunde bei Diabetikern. *Medizinische, 1,* 12, 1959.
4. Arrenvall, L., Eeg-Olofsson, O. and Säwe-Söderberg, J.: Skin biopsy in diabetic children. (To be published.)
5. Bischoff A.: *Die diabetische Neuropathie.* Georg Thieme-Verlag, Stuttgart, 1963.
6. Cohen, C. and Hildebrand J. Latent neuropathy in diabetes and alcoholism. *Neurology 14,* 10, 1965.
7. Downie, A. W and Newell, D. J Sensory nerve conduction in patients with diabetes mellitus and controls. *Neurology 11* 876, 1961.
8. Eliasson S. G and Hughes, A. H.: Cholesterol and fatty acid synthesis in diabetic nerve and spinal cord. *Neurology 10* 143, 1960.
9. Eliasson S. G. Nerve conduction changes in experimental diabetes. *J Clin Invest, 43.* 2353, 1964.
10. Evers, B.: Diabetes und Hirnstromaktivität. *Nervenarzt, 26* 247 1955.
11. Fagerberg S. E.: *Diabetic neuropathy* *Acta Med Scand,* Suppl. *345* 1959
12. Fagerberg, S. E., Petersen, L, Steg, G and Wilhelmsen, L.: Motor disturbances in diabetes mellitus. A clinical study using electromyography and nerve conduction velocity determination. *Acta Med Scand, 174.* 711 1963.
13. Field R. A. and Adams, L.: Insulin response in vitro of peripheral nerve from normal and alloxan diabetic rabbits in parabiosis. *5th Congress Int Diab Fed, Toronto, 1964 1 vol. Excerpta Medica, Edit 84, 1964*
14. Gamstorp I.: *Normal conduction velocity* of ulnar median and peroneal nerves in infancy childhood and adolescence. *Acta Paediat Scand,* Suppl. *146* 68, 1963.
15. — Conduction velocity of peripheral motor nerves in mental retardation, diabetes and various neurological diseases in childhood. *Acta Paediat Scand, 53* 406, 1964.
16. Gassel, M. M.: Sources of error in motor nerve conduction studies. *Neurology 14* 825, 1964.
17. Gibbs, E. L. and Gibbs, F A. Electroencephalographic evidence of thalamic and hypothalamic epilepsy *Neurology 1.* 136, 1951.
18. Gilliatt, R. W and Willison R. G Peripheral nerve conduction in diabetic neuropathy *J Neurol Neurosurg Psychiat, 25* 11 1962.
19. Greenbaum, D Observations on the homogeneous nature and pathogenesis of diabetic neuropathy *Brain, 87* 215, 1964.
20. Greenblatt M., Murray J and Root, H. F Electroencephalographic studies in diabetes mellitus. *New Eng J Med, 234:* 119 1946.
21. Helmholtz, H.: *Wissenschaftliche Abhandlungen.* J. A. Barth, p. 939 1883.
22. Henriksen J D *Conduction velocity of motor nerves in normal subjects and patients with neuromuscular disorders.* Thesis, Mayo Foundation, 1956.
23. Hersley K. M., Karlsson B. and Stersky G EEG studies on cases of juvenile diabetes mellitus of long duration. *Acta Paediat Scand,* Suppl. *138* 57 1962.
24. Hodes, R., Larrabee, M. G and German W The human electromyogram in response to nerve stimulation and the conduction velocity of motor axons. Studies on normal and on injured peripheral nerves. *Arch Neurol Psychiat, 60* 340, 1948.
25. Hoffman J Peripheral neuropathy in children with diabetes mellitus. *Acta Neurol Scand,* Suppl. *4,* 1964.
26. Izzo J L., Schuster, D B and Engel, G L.: The electroencephalogram of patients with diabetes mellitus. *Diabetes, 2.* 93, 1953.
27. Johnson E. W and Olsen K. J Clinical value of motor nerve conduction velocity determination. *J AMA, 172.* 2030, 1960.
28. Jordan W R. Neuritic manifestations in diabetes mellitus. *Arch Intern Med, 57* 307 1936
29. Kellaway P Crawley J W and Kagawa, N A specific electroencephalographic correlate of convulsive equivalent disorders in children. *J Paediat, 55* 582, 1959
30. Keen H.: Autonomic neuropathy in diabetes mellitus. *Postgrad Med J 35.* 272, 1959.
31. Lawrence, D G and Locke, S.: Motor nerve conduction velocity in diabetes. *Arch Neurol, 5* 483, 1961
32. — Neuropathy in children with diabetes mellitus. *Brit Med J 1* 784 1963.
33. Martin M. M.: Diabetic neuropathy *Brain, 76* 594, 1953.
34. Mayer, R. F Nerve conduction studies in man. *Neurology 13.* 1021, 1963.
35. Mulder, D. W Lambert E. H., Bastron J A. and Sprague, R. G The neuro-

pathies associated with diabetes mellitus. A clinical and electromyographic study of 103 unselected diabetic patients. *Neurology 11:* 275, 1961
36. PETERSÉN, L., EEG-OLOFSSON, O., HAGNE, I. and SELLDÉN, U.: EEG in normal children. (To be published.)
37. PLUM, F.: *The Neurologic Complications of Diabetes Mellitus.* Diabetes, Williams ed., Hoeber p. 602, 1960.
38. REFSUM, S., PRESTHUS, J., SKULSTAD, A. and ÖSTENSJÖ, S.: Clinical correlates of the 14 and 6 per second positive spikes. *Acta Psychiat Neurol Scand 35:* 330, 1960.
39. RUDY, A. and EPSTEIN, S. H.: Review of one hundred cases of diabetic neuropathy followed from one to ten years. *J Clin Endocrin, 5:* 92, 1945.
40. SELLDÉN, U.: Electroencephalographic activation with Megimide in normal subjects. *Acta Neurol Scand,* Suppl. *12:* 1964.
41. SIMPSON, J. A.: Fact and fallacy in measurement of conduction velocity in motor nerves. *J Neurol Neurosurg Psychiat 27:* 381, 1964.
42. SKILLMAN, T. G., JOHNSON, E. W., HAMWI, G. J. and DRISKILL, H. J.: Motor nerve conduction velocity in diabetes mellitus. *Diabetes, 10:* 46, 1961
43. SKORPIL, V.: Conduction velocity of human nerve structures. *Rozpr Česk Akad Věd,* 75, 1965.
44. STEINESS, I.: Diabetic neuropathy. Vibration sense and abnormal tendon reflexes in diabetes. *Acta Med Scand* Suppl. *394:* 1963.
45. THOMAS, J. E. and LAMBERT, E. H.: Ulnar nerve conduction velocity and H-reflex in infants and children. *J Appl Physiol, 15:* 1, 1960.
46. WILSON, D. R.: Electroencephalographic studies in diabetes mellitus. *Canad Med Ass J 65:* 462, 1951.

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Deficiency of Pyruvate Kinase in the Erythrocytes of a Child with Hereditary Non-Spherocytic Hemolytic Anemia

by D. BUSCH, L. WITT, M. BERGER and W. KÜNZER with the assistance of K. SCHMUCK and H. MÜLLER

Valentine and coworkers [33, 34] first described pyruvate kinase deficiency in the erythrocytes of patients suffering from hereditary non-spherocytic hemolytic anemia. Since then more than 60 similar cases have been reported [10].

The clinical features of this disease have often been described [11, 17]. The clinical symptomatology of "atypical anemias" characterized by a deficiency of pyruvate kinase does not allow an etiologic identification of this disorder. Also the results of autohemolysis test do not give any reliable indication of etiology because the deficiency of pyruvate kinase is found both in anemias of Type II of Selwyn and Dacie [31] and in anemias of Type I [.., 8].

Biochemical studies [7, 8, 9, 20, 21, 30] have shown that the red cells besides having a reduced activity of pyruvate kinase, are characterized by a reduction of ATP[1] content and an increase of the substrates PEP, 3-PG and 2,3-DPG. This suggests a disturbance in the energy metabolism of the erythrocytes. The decrease of the ATP level may be responsible for the increased hemolysis of the red cells. Usually this type of hemolytic anemia becomes obvious if deficiency of the enzyme in erythrocytes occurs homozygously [32]. Persons who are heterozygous for the gene show an activity of pyruvate kinase which is reduced to nearly half of the normal level but they are clinically healthy. The discrepancy between the seriousness of the hemolytic anemia and the degree of pyruvate kinase deficiency in the erythrocytes in many cases leads us to question whether increased hemolysis can fully be explained by the decrease of the activity of the enzyme. It is the purpose of this communication to report a case of hereditary non-spherocytic hemolytic anemia in a nine-year-old boy which is interesting in this context.

Prof. Dr. H. Hungerland, Director of the Pediatric Clinic of the University of Bonn, to his 60th birthday.

[1] *Abbreviations.* ATP adenosine triphosphate; ADP adenosine diphosphate; AMP adenosine monophosphate; 2,3-DPG 2,3-diphosphoglycerate; 3-PG, 3-phosphoglycerate; 2-PG 2-phosphoglycerate; PEP phosphoenolpyruvate; GSH, glutathione reduced; GSSG glutathione oxidized.

Material and Methods

The biochemical reagents were purchased from C. F. Boehringer u. Söhne GmbH (Mannheim), other analytical grade reagents were obtained from E. Merck AG (Darmstadt) and Riedel-de Haën AG (Seelze-Hannover).

Preparation of erythrocytes and hemolysates: 5 ml venous blood was mixed with 0.5

ml of a solution of 1% ethylenediaminotetraacetate in 0.7% NaCl solution. The erythrocytes were separated by centrifugation at 1000 g for 10 min, leucocytes were discarded and red cells washed three times with 0.9 NaCl solution. They were suspended in a final volume of 3 ml. One ml of the erythrocyte suspension, 1 ml distilled water 0.7 ml 0.05 M triethanolamine buffer pH 7.5, and 0.3 ml saturated digitonin solution were added.

After standing in an ice-bath for 30 min and centrifugation for 10 min at 1000 g the activities of the enzymes were assayed in the supernatant solution.

Assay methods

Enzyme activities Enzyme activities were measured spectrophotometrically. Hexokinase was assayed according to Grignani and Löhr [15], fructose-6-phosphate kinase according to Ling and coworkers [23], 3-phosphoglyceraldehyde dehydrogenase according to Delbrück and coworkers [14], 3-phosphoglycerate-1-kinase according to Bucher [6], pyruvate kinase according to Negelein [26], lactate dehydrogenase according to Kubowitz and Ott [22], glucose-6-phosphate and 6-phosphogluconate dehydrogenase according to Kornberg and Horecker [20] glutathione reductase according to Horn [19], isocitrate dehydrogenase according to Ochoa [27], malate dehydrogenase according to Bergmeyer and Bernt [1].

Metabolite concentrations. The concentrations of metabolites and the rate of glycolysis were measured as described previously [8]. GSH stability was measured according to Beutler [4], GSSG according to Günther berg [18] and 2,3-DPG according to Krimsky [21]

Clinical Symptomatology Chemical Blood Analysis and Hematological Results

Six persons were investigated the 8-year-old patient (J S.) with clinical symptoms, his brothers (Th. S. and S. S.) the parents of the children (T S. and L. S.) and the maternal grand mother

Patient. J S is the 5th child born to a family in which no serious diseases have been known The parents are related to each other (with consanguinity of the 3rd degree). The first two children of the couple both had very severe icterus at birth and died within a few hours. Both succeeding brothers have been healthy until the present. The patient was born spontaneously 3 weeks before the calculated day with a birth weight of 3450 g and a length of 51 cm. Because of icterus with a bilirubin level of 32 mg/100 ml of serum and a decrease of the hemoglobin to 0 0 g/100 ml, an exchange transfusion was performed on the first day of his life. Serologically there was no evidence for a sensitization in blood group systems. The icterus subsided very slowly but reappeared in the 5th week.

At the age of six months he received a blood transfusion because of severe anemia of unknown etiology The further development of the child was not remarkable, until he entered our clinic because of slight icterus and easy fatigability when he was 8 years old. The boy was then normally developed both physically and mentally Scleral icterus was evident. The spleen was palpable one half inch under the costal margin. Other abnormalities could not be found on physical examination. Hematological results are listed in Table 1 together with those of the other subjects studied.

Further results in patient J S. The direct Coombs test was negative Cold-agglutinin titer was normal (1 8). The acidified-serum test (HAM's test) did not

TABLE 1 *Results of hematological investigations*

Person	Hb g %	MCH	MCD (μ)	Reticulocytes, %	Haematocrit, %	Bilirubin mg %	Bilirubin "Indirect" mg %	Osmotic fragility	HbA %	HbF	Haptoglobin
G. L.	12.1	31.8	8	1.0	37.5	—	—	Normal	1.44	0.14	Normal
T. S.	13.6	34.6	8	1.0	46	—	—	Normal	—	—	Normal
L. S.	13.4	31.8	8	2.0–6.0	40	0.3	—	Normal	0.78	1.7	Normal
Th. S.	17.5	32.7	8	1.8	41	—	—	Normal	0.96	0.84	Normal
J. S.	10.7	34.3	8	2.0–16.0	31	2.2	1.71	Normal	0.86	2.2	Decreased

X Icterus observed.

indicate an alteration in the erythrocytes of the PNH type. The blood group was B Rh positive. In the bone-marrow there was an increase of all erythrocytic elements.

Autohemolysis test according to Selwyn and Dacie [31]. Without addition: 4.0 % (normal 1.0–3.5 %) with glucose: 3.0 % (normal 0.0–0.7 %); with ATP: –6 % (normal 0.0–0.8 %).

The serum content of iron was 55 μg/100 ml, and of copper 94 μg/100 ml. Activities of transaminases and alkaline phosphatase were normal, also the electrophoretic mobilities of the serum proteins.

Family: When the family was studied the brothers of the patient were aged 10 and 12 years. The elder of the two brothers, Th. S. had icterus at birth. This however subsided quickly. All other persons examined denied any serious diseases. The clinical examination of Th. S. and S. S. did not show any peculiarities. Both children were developed according to their age and there was neither icterus nor splenomegaly.

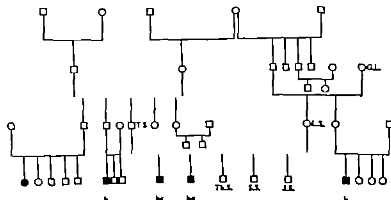


Fig. 1 In the generation of the patient, there is a striking frequency of abortions and deaths of newborn children. The common grandmother of the parents should be particularly emphasized. So far as the relatives could remember, she had diabetes, but never suffered from anemia or icterus. a, Abortion; b died after birth; c, icterus at birth.

TABLE 2. *Enzyme activities in erythrocytes (μ moles substrate converted per min per 10[11] cells)*

Enzyme	Normal values[b] Mean	Normal values[b] ±1 s.d.	J. S. (Patient May 1964)	J. S. (Patient July 1964)	Th. S. (Brother)	S. S. (Brother)	T. S. (Father)	L. S. (Mother)	G. L. (Mother's mother)
Hexokinase[a]	1.3	0.6	—	[illegible]	0.8	0.8	2.0	1.7	1.1
6-Phosphofructokinase	23.1	2.4	—	23.0	43.2	37.0	—	22.4	28.0
Glyceraldehyde-3-phosphate dehydrogenase	285.0	50.0	—	312.0	308.0	[illegible]	314.0	292.0	303.0
3-Phosphoglycerate kinase	218.0	45.8	228.0	252.0	200.0	40.0	300.0	224.0	274.0
Pyruvate kinase	4.6	6.7	9.3	11.0	23.4	19.0	8.2	21.9	34.9
Lactate dehydrogenase	211.0	[illegible]	[illegible]	237.0	187.0	195.0	—	[illegible]	40.0
Glucose-6-phosphate dehydrogenase	15.8	3.9	26.3	10.6	15.3	17.3	14.8	16.0	16.8
6-Phosphogluconat dehydrogenase	10.0	1.7	13.8	11.7	7.0	8.9	6.0	9.4	10.9
Glutathione reductase TPNH-dependent	14.1	4.1	—	15.6	18.0	18.3	[illegible]	1.0	21.4
Glutathione reductase DPNH-dependent	8.3	[illegible]	—	8.2	[illegible]	9.3	6.6	10.3	11.3
Isocitrate dehydrogenase[a]	3.0	0.4	—	3.2	2.8	2.9	—	2.7	3.0
Malate dehydrogenase	349.0	114.0	418.0	420.0	312.0	318.0	—	437.0	448.0

[a] Hexokinase and isocitrate dehydrogenase were measured at 37°C. All other enzymes were measured at 25°C.

[b] Measured in the biochemical laboratory of the Pediatric Clinic.

TABLE 3. *Concentrations of red cell compounds.*

Values are expressed as mμ-moles per ml of packed fresh red cells except for lactate and pyruvate, which are expressed as mμ-moles per ml blood.

	Mean range[a] Mean ± 2 s.d.	J. S. (Patient)	Th. S. (Brother)	S. S. (Brother)	T. S. (Father)	L. S. (Mother)
2,3-DPG	2500 (2000–3000)	4160	2020	1440	4770	2570
ATP	1280 (1030–1530)	770	1080	1310	1140	1200
ADP	279 (165–396)	178	242	234	320	226
AMP	51 (10–92)	56	53	52	53	46
ATP/ADP	4.9 (2.7–7.1)	4.3	4.5	3.6	3.6	5.3
Lactate	1450 (520–2380)	1140			1180	
Pyruvate	134 (40–228)	86	123	109	94	197
Lactate/Pyruvate	11.3 (6–17)	13			12.3	

[a] Mean range see Busch, 1964 (8).

Biochemical Results

The results of the biochemical investigations in the red cells of the patient and the family are shown in Tables 2–5

Discussion

The patient, a 9-year-old boy was found to be suffering from a hereditary nonspherocytic hemolytic anemia. Both parents, related to each other were clinically healthy Two siblings had died with icterus *immediately after birth*. Two elder brothers were clinically healthy

Estimation of the activities of several enzymes in the erythrocytes of the diseased child showed a decreased activity of pyruvate kinase (Table 2) *This was not a* very marked decrease, being in the same range as is normally observed in persons heterozygous for this disorder We found also a decreased activity of pyruvate kinase in the erythrocytes of the boy's father This decrease was slightly more marked than that of the child. In the erythrocytes of the mother and both brothers, the pyruvate kinase activities were at the lowest limit of the normal range

TABLE 4. *Glyceric acid and phosphoenol pyruvate concentrations from patient J S*

Values are expressed as mμ-moles per ml of packed fresh red cells.

	Mean range* Mean ± 2 s.D.	J S.
3-PG	7* (33–111)	127
2 PG	30 (10–50)	19
PEP	21 (9–33)	63

Mean range see Busch, 1964 [8].

TABLE 5. *Rate of glycolysis in fresh red cells from patient J S*

pH 7.2, t = 37°

	Normal controls*	J S.
Lactate formation from glucose (mμ-moles per ml red cells per min.)	30–50	60

N = 10.

The mode of transmission of pyruvate kinase deficiency is autosomal. According to common assumption, heterozygous carriers of the trait, generally showing about half the normal pyruvate kinase activity are clinically healthy [32]. The question arising in our case, whether it is a heterozygous pyruvate kinase deficiency *associated with a hemolytic jaundice or a* homozygous deficiency whose peculiarity lies in the relatively high pyruvate kinase activity in the erythrocytes (nearly 50% of the mean value), cannot be answered. *The results of activity estimations of the* deficient enzyme do not allow to decide this problem, as is seen for instance in cases of Bestetti [2] and Oski and Diamond [28] too

However it is an interesting fact that in our patient a relatively small biochemical abnormality is associated with such a marked increase of hemolysis. In contrary the father shows no abnormal clinical *symptoms, despite the enzyme deficiency* which is more pronounced than in the child. Discrepancies between the gravity of the clinical symptoms and the degree of pyruvate kinase deficiency in *the erythrocytes have already been mentioned* [9, 13, 28]. It is difficult to explain the increased hemolysis only by the slight

reduction of pyruvate kinase activity although pyruvate kinase is a rate limiting enzyme in the glycolysis of erythrocytes [7 9] Furthermore we assume that the augmentation of PEP effects a stimulation of activity of the enzyme in the deficient cells, which is considerably under saturated. Accordingly we found a normal rate of glycolysis (Table 5)

Therefore the importance of additional disturbances in the abnormal red cells must be discussed. It is evident, that the activity of the glucose-6-phosphate dehydrogenase (G 6-PD) (Table) is relatively low considering the existent reticulocytosis. Identical results have been obtained in other pyruvate kinase deficient cases [5 9] Rapoport [29] has described two children with pyruvate kinase deficient atypical hemolytic anemia with an increased G-6-PD lability (in vitro) Moreover Brunetti and coworkers [5] found in their cases a decreased concentration of pentoses in the erythrocytes. Busch [7] described in one case an increased level of GSSG in the red cells and a reduction in the content of NAD and NADH the last recently confirmed by De Gruchy and coworkers [17].

We have no explanation for these results up to now Our patient showed normal levels of GSH and GSSG and the stability of reduced glutathione after incubation of the erythrocytes with acetyl phenylhydrazine (Beutler test [3]) was normal. Further studies in this case have to be performed.

Investigations of the glycolytic intermediates in the red cells showed the same deviations as reported for other cases of pyruvate kinase deficiency decrease of ATP as well as an increase of 2,3-DPG 3-PG and PEP [9] In the erythrocytes of the elder brothers we did not find any pathological alterations. There were also no abnormal clinical findings.

The reported anemia belongs to "Type I" according to Selwyn and Dacie [31]. In most cases the pyruvate kinase deficiency is found in anemias of Type II [12]. However anemias of "Type I" with pyruvate kinase deficiency have already been described [2, 7]. Also some cases published by Oski [28], Brunetti [5], and Tanaka [3_] may be considered to Type I. The results of the autohemolysis test do not allow a clear classification in respect to the biochemical background of the disease

Summary

We report on a nine year-old boy with the symptoms of a hereditary non-spherocytic anemia, whose erythrocytes showed a decrease of pyruvate kinase activity to about 50 % of the normal level A low level of ATP and an increase of the concentrations of 2,3-DPG 3-PG and PEP were also found. Genetical and biochemical problems are discussed. This anemia belongs to Type I according to Sel yn and Dacie

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References

1. BERGMEYER, H. U and BERNT, E.: Malat-dehydrogenase. In: *Methoden der enzymatischen Analyse*. Ed.: H. U BERGMEYER, Verlag Chemie 1962, p. 757
2. BERTRETTI, A., ROSSI, U LOOS, J A. and PRINS, H. K.: A case of congenital atypical haemolytic anaemia with pyruvate kinase deficiency *Vox Sang, 9* 492, 1964.
3. BEUTLER, E., ROBSON M. J and BUTTENWIESER, E.: The glutathione instability of drug-sensitive erythrocytes. A new method for the in vitro detection of drug sensitivity *J Lab Clin Med, 49* 84, 1957
4. BEUTLER, E., DURON, O and KELLY B. M.: Improved method for the determination of blood glutathione. *J Lab Clin Med, 61* 882, 1963.
5. BRUNETTI, P PUXEDDU A., NENCI, G and MIGLIORINI, H.: Congenital non-spherocytic haemolytic anaemia due to pyruvatekinase deficiency *Acta haemat 30* 88, 1963.
6. BÜCHER, TH.: Über ein phosphatuber tragendes Gärungsferment. *Biochim Biophys Acta 1* 292, 1947
7. BUSCH, D Erythrocyte metabolism in three persons with hereditary nonspherocytic hemolytic anemia, deficient in pyruvate kinase. *Proc. 9th Congr Europ. Soc. Haemat., Lisbon 1963*, p. 783 S. Karger, Basel/New York 1963.
8. — Kongenitale nichtsphärocytäre hämolytische Anämie mit Mangel an erythrocytärer Pyruvatkinase. *Folia haemat, NF 9* 90, 1964.
9. BUSCH, D. and SCHRÖDER, K.: Probleme des Erythrocytenstoffwechsels bei Anämien mit Pyruvatkinase-Mangel. *Folia haemat*, Leipzig, 1965. In press.
10. BUSCH, D Hereditäre nichtsphärocytäre hämolytische Anämien. In: *Handbuch der inneren Medizin, Band 2, Blut und Blutkrankheiten*. Ed L. HEILMEYER, Springer Berlin–Göttingen–Heidelberg 1965. In press.
11. DACIE, J V *The Hemolytic Anemias. Part I The congenital anemias*. 2nd ed. Churchill, London 1960
12. — The hereditary non-spherocytic haemolytic anaemias. Proc. 9th Congr Europ. Soc. Haemat. Lisbon 1963. *Acta haemat, 31* 177 1964.
13. — Pers comm
14. DELBRÜCK, A., ZEBE, E. and BÜCHER, TH.: Über Verteilungsmuster von Enzymen des energieliefernden Stoffwechsels im Flugmuskel, Sprungmuskel und Fettkörper von Locusta migratoria und ihre cytologische Zuordnung. *Biochem Z, 331* 273, 1959.
15. GHIGNANI, F and LÖHR, G. W Über die Hexokinase in menschlichen Blutzellen. *Klin Wschr 1960* 796.
16. GRIMES, A. J., MEISLER, A. and DACIE, J V Hereditary nonspherocytic haemolytic anaemia. A study of red-cell carbohydrate metabolism in twelve cases of pyruvatekinase deficiency *Brit J Haemat, 10* 403, 1964.
17 DE GRUCHY G. C., SANTAMARIA, J N PARSONS, I. C. and CRAWFORD H.: Nonspherocytic congenital hemolytic anemia. *Blood, 16* 1371, 1960.
18. GUTTENBERG, H. and ROST, J Bestimmung von GSSG in roten Blutzellen. *Folia haemat*, Leipzig, 1965. In press.
19. HORN H. D Glutathionreduktase. In: *Methoden der enzymatischen Analyse*. Ed.: H. U BERGMEYER, Verlag Chemie 1962, p. 875.
20. KORNBERG, A. and HORECKER, B. L. Glucose-6-phosphate dehydrogenase. *Methods in Enzymology 1* 323, 1955.
21. KRIMSKY I.: D-glycerat-2,3-diphosphat *Methoden der enzymatischen Analyse*. Ed.: H. U BERGMEYER, Verlag Chemie 1962, p. 238.
22. KUBOWITZ, F and OTT, P Isolierung und Kristallisation eines Gärungsfermentes aus Tumoren. *Biochem Z 314* 94, 1943.
23. LING, K. H., BYRNE, W L. and LARDY H.: Phosphohexokinase. *Methods in Enzymology, 1* 306, 1955.
24. LÖHR, G W and WALLER, H. D Zur Biochemie einiger angeborener hämolytischer Anämien. *Folia haemat, NF 8* 377 1963.
25. LOHMER, P B. and DE GRUCHY G C.: Red-cell enzymes and co-enzymes in non-spherocytic congenital haemolytic anaemias. *Brit J Haemat, 11* 21 1965.
26. VOGELL, E. Zn. nach BERGMEYER, G., BOLTZE, H. J BÜCHER, TH., CZOK, R., GARBADE, K. H., MEYER-ARENDT, E. and PFLEIDERER, G Diphosphofruktose-Aldolase, Phosphoglycerinaldehyd-Dehydrogenase, Milchsäure-Dehydrogenase, Glycerophosphat-Dehydrogenase und Pyruvatkinase aus Kaninchenmuskel in einem Arbeitsgang. *Z Naturforsch, 8b* 555, 1953.
27 OCHOA, S.: Biosynthesis of tricarboxylic acids by carbon dioxide fixation. III. Enzymatic mechanisms. *J Biol Chem, 174* 133, 1948.
28. OSKI, F A. and DIAMOND, L. K.: Erythrocyte pyruvate kinase deficiency resulting in congenital nonspherocytic hemolytic anemia. *New England Journal Med, 269* 763, 1963.
29 RAPOPORT, S. M. in Gemeinschaftsarbeit des Physiologisch-Chemischen Institutes Berlin und der Univ Kinderkliniken Berlin und

reduction of pyruvate kinase activity although pyruvate kinase is a rate limiting enzyme in the glycolysis of erythrocytes [7, 9]. Furthermore we assume that the augmentation of PEP effects a stimulation of activity of the enzyme in the deficient cells, which is considerably undersaturated. Accordingly we found a normal rate of glycolysis (Table 5).

Therefore the importance of additional disturbances in the abnormal red cells must be discussed. It is evident that the activity of the glucose-6-phosphate dehydrogenase (G 6-PD) (Table ?) is relatively low considering the existent reticulocytosis. Identical results have been obtained in other pyruvate kinase deficient cases [5, 9]. Rapoport [29] has described two children with pyruvate kinase deficient atypical hemolytic anemia with an increased G-6-PD lability (in vitro). Moreover Brunetti and coworkers [5] found in their cases a decreased concentration of pentoses in the erythrocytes. Busch [7] described in one case an increased level of GSSG in the red cells and a reduction in the content of NAD and NADH, the last recently confirmed by De Gruchy and coworkers [17].

We have no explanation for these results up to now. Our patient showed normal levels of GSH and GSSG and the stability of reduced glutathione after incubation of the erythrocytes with acetylphenylhydrazine (Beutler test [3]) was normal. Further studies in this case have to be performed.

Investigations of the glycolytic intermediates in the red cells showed the same deviations as reported for other cases of pyruvate kinase deficiency: decrease of ATP as well as an increase of 2,3-DPG, 3-PG and PEP [9]. In the erythrocytes of the elder brothers we did not find any pathological alterations. There were also no abnormal clinical findings.

The reported anemia belongs to "Type I" according to Selwyn and Dacie [31]. In most cases the pyruvate kinase deficiency is found in anemias of "Type II" [12]. However anemias of "Type I" with pyruvate kinase deficiency have already been described [2, 7]. Also some cases published by Oski [28], Brunetti [5], and Tanaka [3.] may be considered to Type I. The results of the autohemolysis test do not allow a clear classification in respect to the biochemical background of the disease.

Summary

We report on a nine-year-old boy with the symptoms of a hereditary non-spherocytic anemia whose erythrocytes showed a decrease of pyruvate kinase activity to about 50% of the normal level. A low level of ATP and an increase of the concentrations of 2,3-DPG, 3-PG and PEP were also found. Genetical and biochemical problems are discussed. This anemia belongs to Type I according to Selwyn and Dacie.

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Staphylococcus Aureus and Infections on Maternity Wards

III *Bacterical Colonization of the Umbilicus and Exchange transfusions*

by B. JALLING R. LAGERCRANTZ, K. E. MYRBÄCK and L. ENGSTRÖM

Earlier studies on the maternity wards of Karolinska Hospital showed 0–83 % of newborns to harbour Staphylococcus aureus (S. aureus) [., 3] Infections, probably caused by these organisms, occurred in 11–12 % of children and/or their mothers. Use of phisoHex for handwashing for personnel and mothers did not materially reduce colonization or infections. Looking for other reservoirs in the newborn we became interested in the bacteriology of the umbilicus. This is of special importance in patients who are exchange transfused via the umbilical vein. Bacteremia and septicemia have been reported [8, 9] as complications of exchange transfusions. Two cases of omphalitis and septicemia with S. aureus (phage type 80/81 and 52/5. A/80/81 respectively) in prematures . days after exchange transfusions via the umbilicus increased our interest in the bacteriology of exchange transfusions. The first part of this report deals with bacteriological examinations of the umbilicus and blood in cases exchange transfused via the umbilicus. The majority of the umbilici were colonized with bacteria before the exchange transfusion. To reduce contamination, we introduced treatment with Nebacetin (a mixture of Bacitracin and Neomycin) as powder or lotion before and between exchange transfusions. The effect of this measure was not striking Spraying of the umbilicus with Nobecutan was recently recommended by Andersen, Holm & Winberg [1]. Results of a controlled study of Nobecutan in an unselected group of newborns are reported in the third and main part of our study

The wards and the nursing routine were previously described [2, 3]. Ordinary soap and paper towels were used for handwashing The personnel did not wear masks. Disposable paper diapers ("TPT" Stille Werner Stockholm) were used which did not cover the umbilicus. The umbilical cord was ligated with a light metal clamp (of Lofset type [10]).

Part 1

Material and Methods

All cases exchange-transfused during October and November in 1962 were examined. At that time the umbilical cords were covered with compresses moistened with quartenary ammonium-solution (Benzethon). Before transfusion the cord was cut. Specimens for culture were taken from the cut surface

TABLE 1 *Bacteriological findings at exchange transfusion in the 10 cases with pos blood cultures.*

Case number	Age (days)	Cultures from umbilicus	Cultures from patient blood before transfusion	Cultures from patient blood after transfusion	Cultures from blood bottle
B 62 09 27	7	S. ureus	Gram neg. rods	—	S. aureus[a]
S 62 09 30	5	—	Enterococci	Enterococci	S. albus[a]
B 62 10 03	1	E. coli	—	—	S. albus[a]
A 62 10 13	4	Gram neg. rods	—	—	S. albus[a]
L 62 10 20	5	S. aureus	S. aureus	—	—
H 6. 10 24	4	S. aureus	S. aureus enterococci	—	—
B 62 11 05	6	S. aureus	S. ureus	S. albus	—
H 62 11 06	0	—	S aureus	—	—
P 62 11 16	6	S. ureus	S. ureus	—	—
W 62 11 29		E. coli	Enterococci	Enterococci	—

[a] See text.

on a moist cotton swab, preserved in Loefflers serum tubes and cultured within 2 hours on blood agar plates for 18 hours at +37 C The exchange transfusion was performed as recommended by Diamond et al. [5]. Immediately after insertion of the umbilical catheter blood was withdrawn from the bottle of blood and from the patient through the transfusion set Another sample was taken from the patient at the end of the transfusion. The growth was quantitated as slight moderate or abundant Only coagulase positive strains were registered as S. aureus. Sensitivity tests against antibiotics were carried out as described by Ericsson et al. [4]. Phage typing was performed at the National Bacteriological Laboratory

Results

In the period under study 43 exchange transfusions were carried out on 28 patients. Culturing was complete in 28 of the transfusions. In 18 of these no bacterial growth was found in the patients blood or in the blood used for the exchange. In only 6 of these 18 cases were pathogenic bacteria obtained from the cord. The bacteriological findings in the 10 cases with positive blood culture are shown in Table 1 The mean age was 4 days in cases with positive blood culture and 2 days in those with negative blood culture

In no case were the same pathogenic bacteria cultured from the transfusion set and the patient. In 6 of 8 cases with pathogenic bacteria found in one of the blood samples, such bacteria were also found in the umbilicus. None of the 8 patients with positive blood cultures had symptoms of sepsis. Positive cultures from the bottle of blood (Table 1) probably represent contaminants from the umbilici and the transfusion set.

Part 2

Materials and Methods

In January and February 1963, the umbilici of all patients admitted to the nursery ward because of hyperbilirubinemia and/or Rh-immunization were continuously covered with Nebacetin as powder for those born on even days, as 1% lotion for those born on uneven days. On the child's arrival to the ward, a specimen was taken from outside the clamped cord, which then was cut and treated. Swabs for bacteriological

culture were taken daily after washing with sterile water. The specimens were handled in the same manner as in Part 1 of this study.

Results

Nebacetin was used in 70 cases, in 36 as powder and in 34 as lotion. No difference was observed between the two groups. The bacteriological results for the whole material are shown in Fig. 1 A and B. A comparison between bacteriological findings from inside and outside of the umbilical cord is given in Table 2. No case of omphalitis or septicemia occurred.

Part 3

Material and Methods

The third part of this study comprised a study of Nobecutan as a spray for the umbilicus. We followed the method described by Andersen et al. with slight modifications: for preservation of the swabs, we used ordinary Loefflers tubes whereas they used a special medium in order to prevent influence of the disinfectant in Nobecutan and growth during transport. All infants born during four weeks in October–November 1963, were studied. The umbilici of the babies born at night were powdered with Xeroform (equal parts of boric acid and bismuth tribromphenolas) which is the routine treatment in our department. The umbilici of infants born during the day were sprayed with Nobecutan. The personnel on the delivery floor alternated day and night. Four to 24 hours after delivery the infants were transferred from the delivery ward to four different wards where once daily the care of the umbilicus was continued in the same way as first instituted. Specimens were taken from the sides and the tip of the cords on moist cotton swabs on delivery, on the second and third day and on the day of discharge (mostly on the sixth day). Before culturing the cords were cleaned with sterile water. The specimens were handled in the same manner as in Part 1 of the investigation.

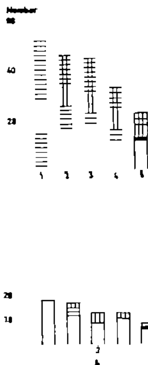


Fig. 1. Umbilical colonization in cases treated with Nebacetin. (a) Cases with colonized umbilicus on arrival. (b) Cases without colonization on arrival. ▦ S. aureus; ▥ pathogenic bact. other than S. aureus; ▤ S. albus and/or other pathogenic bact.; ☐ neg. cult. 1–3 days after institution of treatment.

Results

No case of clinical omphalitis was observed during the study period. The bacteriological results of the controlled trial of Nobecutan are presented in Fig. 2 and Tables 3–6. There was no significant difference between the group where Nobecutan was used and the controls. (Fig. 2 $\chi^2 = 1$.—not significant at 5% level.) S. aureus was isolated in the same frequency

TABLE 2. *Comparison between bacteriological findings from inside and outside the umbilical cord.*

		Culture from inside the umbilical cord after cutting		
		Pos.	Neg.	Total
Culture from outside the cord	Pos.	33	6	39
	Neg.	8	3	11
Total		41	9	50

TABLE 3. *Bacterial growth and care of the umbilicus*

Growth of one or more strains	Conventional care	Nobecutan
Staf. albus	33	48
Staf. aureus	34	45
E. coli, coliforms Proteus, Pyocyanus, β-hemolytic streptococci, fungi	41	63
Four or more strains	4	6
Rate of cases with bacteria	45/45	0/71

TABLE 4. *First isolation of S. aureus related to care of umbilicus*

Day after delivery when S. aur. appeared	Growth of S. aureus in the umbilicus: Conventional care	Nobecutan
0	0	0
1	21	28
2	9	8
6–7	4	9
Rate of cases with S. aureus	34/45	45/71



Fig. 2. Colonization of the umbilicus in cases treated with conventional method versus method with Nobecutan. ▥ S. aureus; ▭ no S. aureus.

in patients on different wards and during different weeks. A possible interference between S. albus and S. aureus is demonstrated in Table 4. Antibiograms and phage patterns of the isolated strains of *S. aureus* are given in Table 5.

TABLE 5. *Interference between Staph. albus and aureus.*

	Growth of S. aureus	No growth of S. aureus
First colonization with S. albus	6	11
First culture with S. albus and α-streptococci	6	3
No other growth	17	1

Discussion

These and earlier studies have shown the maternity wards of Karolinska Hospital to be heavily contaminated by bacteria, notably S. aureus. Umbilical cords were found to be a common reservoir for *these bacteria, as shown in many earlier* studies [4, 10, 11]. It has been demonstra-

TABLE 6. *Phage type and antibiogram for 85 strains of S. aureus.*

Phage type with	Sensitive to: Sulfa	Penicillin	Methicillin	Erythromycin	Streptomycin	Tetracyclin	Chloramphenicol	Number
80/81	8	8	9	9	9	8	9	10
53/83 A	5	0	6	6	4	4	6	6
47	6	1	7	7	7	7	7	7
83	4	0	4	4	4	4	4	4
Other	11	8	14	14	12	11	14	15
Non typable	33	24	42	43	39	38	43	43
% sensitive	78	44	97	97	88	86	97	

ted that exchange transfusions through the umbilicus later than three days after delivery carry a risk for septicemia [8, 9]. At this time most navels in our series were colonized. However, in view of the fact that repeated exchange transfusions are often needed, we have not changed our route of transfusion. Other routes, e.g. through supra-umbilical incision, have been recommended [8]. These routes may be less accessible than the umbilicus. Repeated exchange-transfusion (often required) via such incisions might also carry a risk of infection.

Even if no clinical infections were observed during this study, the wide-spread contamination warranted measures. Antibiotics for local use have been shown to diminish infection [10]. We chose Nebocetin because it cannot be given as systemic therapy and most strains were sensitive in vitro to at least one of its components. Nebocetin as powder or lotion did not, however, sterilize the umbilici, but possibly prevented sterile ones from becoming colonized (Fig. 1a and *b*). That many cords remained sterile might, however, be due to some factor other than the treatment with antibiotics. Even without these drugs, they may have been a less favourable milieu for contamination.

As the results with Nebocetin were not encouraging, we tried Nobecutan. This was not significantly more effective than our ordinary treatment with Xeroform—the difference was not significant at the 5% level. This differs from the results of Andersen et al. Differences in technique mentioned earlier can influence the comparison between Nobecutan and controls between their and our results, but probably not the comparison *within* the studies. It is possible that the difference is due to the higher incidence of contamination among our patients than among those of Andersen et al.—this can however not be proved as the bacteriological techniques differ. In a recent study from Denmark, Nobecutan spray did not lower the incidence of colonization [10]. In that study Nobecutan was, however, used only after delivery, which in Andersen's et al. study was not effective.

Most strains were sensitive to most common antibiotics, reflecting our restricted use of these drugs (Table 6). We are anxious to preserve this practice and do not plan to start prophylaxis with anti-

biotics at exchange transfusions With such prophylaxis, hospital infections cannot be prevented but are only made less accessible to antibiotic therapy

These attempts to decrease colonization have not been successful New measures can be discussed. Bathing of the newborns with hexachlorophene and a very strict hand hygiene have been effective in other maternity wards [3 6]. However conta mination per se can but probably should not be prevented. In this study early colonization with S albus seems to some extent to protect against colonization with S. aureus (Table 4) This is an interesting example of bacterial interference A nasal cream with S. albus has earlier been shown to protect against nasal colonization with S. aureus [7] The object is to prevent epidemic spread of virulent strains. With all newborns on a ward cared for by the same personnel in the same room an epidemic can easily occur If mothers and their children were together in small units ("rooming in") and the babies mostly nursed by their mothers, this risk would be considerably smaller

Summary

Bacteriological examinations of the um bilici, blood transfusion-sets and patients blood were performed at 28 exchange transfusions via the umbilicus 0-7 days *after delivery Most of the umbilici were* colonized with bacteria at the time of exchange. No case of septicemia or omphalitis occurred.

Nebacetin as powder or lotion did not sterilize the cords but possibly protected sterile cords from becoming infected.

Daily spray with Nobecutan did not protect the umbilici from becoming conta minated. The effect on the infection of Nobecutan was not better than that of Xeroform powder No clinical colonization occurred. Contamination with S. albus *may have protected against colonization* with S. aureus. Most strains were sensitive to most of the common antibiotics.

References

1. Andersen H. J Holm, S. E. and Winberg, J Bakterieväxten i nyföddas na lar vid två olika antiseptiska behandlingsmetoder (Bacteria in the umbilici of newborns with two different antiseptic methods of care.) *S Läkartidn, 61* 99, 1963.
2. Cederberg B., *Lagercrantz, R.* and Nordén L.: Infections and Staphylococci on the maternity ward. I. *Acta Paediat Scand, Suppl 135* 45, 1962.
3. Cederberg B., Lagercrantz, R. and Nyström, L.: Infections and Staphylococci on the maternity wards. II. *Acta Paediat Scand, 53.* 230, 1964.
4. Ericsson H., Högman, C. and Wickman K.: A paper disc method for determination of bacterial sensitivity to chemotherapeutics and antibiotic agents. *Scand J Clin Lab Invest 6 Suppl 2.* 21 1954.
5. Diamond L. K., Allen F H. and Thomas, W O.: Erythroblastosis fetalis. VII. Treatment with exchange transfusion. *New Engl J Med, 244* 39, 1951
6. Gezon, H., Thompson D Rogers, H., Hatch, T and Taylor, P Hexachlorophene bathing in early infancy *New Engl J Med, 270* 379 1964
7 *Gould, J C* The ecological approach to hospital infections. *Scot Med J 9* 66, 1964.
8. Holm, S E., Lind D and Waldenström, J Tre fall v stafylokocksepsis hos nyfödda efter utbytestransfusion. (Three cases of staphylococco-sepsis in newborns after ex hange transfusions.) *Sv Läkartidn, 58* 1832, 1961
9. Johnston R. and Sell, S.: Observations on blood cultures with exchange transfusion of the newborn infant. *Pediatrics, 27* 1007 1961.
10. Laursen H., Feldo, M. and Rasmussen E: Bakteriologiske undersøgelser ved forskellige na lingsmetoder (Bacteriological

examinations in different care of the umbilicus.) Nord Med, 69 737 1963.

11. Love, G. Gezon, G. Thompson, D. Rogers, K. and Hatch, T. Relation of intensity of staphylococcal infection in newborn infants to contamination of nurses hands and surrounding environment. *Pediatrics*, 32 956, 1963.

12. Mortimer, E., Lipsitz, P. Wolinsky, E., Gonzaga, A. and Rammelkamp, C.: Transmission of Staphylococci between newborns. *New Engl J Med* 26 11., 196..

13. Sanchez, F. R.: A new approach to catheterization of the umbilical vein for exchange transfusion. *Pediatrics*, 25 485, 1960.

14. Wolinsky, E., Gonzaga, A. and Mortimer, E.: The mother as a source of neonatal staphylococci. *New Engl J Med* 267 535, 196..

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Craniosynostosis as a Complication after Operation for Hydrocephalus

by HUGO ANDERSSON

With the introduction of the new methods of using ventriculo-venous shunts for operation of hydrocephalus, the interest in hydrocephalus surgery has increased considerably. The fairly good results have even diminished the previous negative attitude against surgery for hydrocephalus. Consequently we operate more and more hydrocephalus and accordingly we see more of the complications.

Currently the predominate method of treatment is the ventriculo-venous shunt either the Spitz-Holter or the Pudenz-Heyer technique. Reports on technical and biological complications are numerous in recent communications [5, 6, 7]. The most common complications of these procedures are blocking of the shunt mechanism, thrombosis of the large vessels and infections of different kinds.

A less known complication is premature synostosis of the cranial sutures. At the time of operation of hydrocephalus the child has a large circumference of the skull and increased intracranial pressure associated with suture diastasis. If left untreated delayed closure of the sutures occurs. However after a successful operation there is diminished intracranial pressure and apposition of the cranial bones. Sometimes there develops a subnormal intracranial pressure and premature closure. This has been briefly mentioned by Dawson, Emery, Matson and Pappas [1, 2, 3, 4].

The present paper intends to report 3 cases with premature synostosis after ventriculo-venous shunts. Two of the cases are children born with myelomeningoceles which after surgical treatment developed hydrocephalus. The third case was a congenital aqueductal stenosis. All 3 cases were operated upon with a Spitz-Holter shunt. It has been occasionally recommended in the literature that a "low pressure" valve be used when operating upon newborn children. Accordingly a "low pressure" valve was in two of the cases used and a "medium pressure" valve in the remaining case.

Case 1. The fourth child to a 33-year-old woman. Pregnancy and delivery without complication. Birth weight 4.2 kg. Head circumference 35 cm. The child was noted to have a lumbosacral myelomeningocele with thin skin covering, the size of an egg, combined with flaccidity of both legs and sphincters with permanent urine flow. No increased fontanel tension.

The myelomeningocele was not treated surgically. The head circumference was thereafter steady progressing and the fontanel became tense. The head circumference was 46 cm at 7 weeks age. The patient was brought to neurosurgical department for treatment. Plain X-ray of the head showed hydrocephalic cranium with marked digital impressions. The bitemporal diameter of the skull was measured to 13 cm. The child was operated with a ventriculo-venous shunt (Spitz-Holter). During the operation the cortical thickness was measured to 1½ cm. Postoperative course was uneventful. Ten days later at the age of two months, the myelomeningocele was treated surgically in the usual way. It was now noted that the cranium circumference had diminished and the edges of the parietal bones were close to each other. New X-ray of the skull 1 month after the ventriculo-venous shunt showed that the bitemporal diameter had diminished from 13 to 11 cm and the sutures were very narrow. The child was transferred to the parents for care.

Eight months old, the child was again brought to hospital for revision of the cardiac end of the ventriculo-venous shunt, which had been retracted above the level of D III. The examination of the child disclosed a typical scaphocephalic deformity of the head. The fontanels were closed and it was impossible to elicit any movement in the sagittal suture when compressing the head. Head circumference was now 47 cm. The legs were no longer flaccid and the child moved spontaneously in both hips and knee joints.

No surgical intervention was planned for the closure of the sagittal suture.

Case 2. First child of a diabetic mother. The delivery was complicated with breech presentation and after 4 days of ineffective labour a caesarian section was done. The child was pale and asphyctic. The weight was 2.8 kg. Head circumference 38.5 cm with classical signs of congenital hydrocephalus. X-ray of the skull showed hydrocephalic cranium with wide suture separation and thin skull bones. Encephalography and ventriculography were done and these investigations showed an aqueductal stenosis with symmetric hydrocephalus and cortical thickness of 1–1½ cm. At 14 days age the child was operated with a ventriculo-venous shunt (Spitz-Holter) and the postoperative course was uneventful. 14 days after the operation it was noted that there was a general apposition of the sutures with unchanged head circumference. A new control at 5 months age showed the same head circumference, 38.5 cm. The cranium was now deformed with palpable ridges on the place of the sutures and the temporal bones were flattened and insunk. An X-ray of the skull this time shows diminished cranium, especially the broad diameter and also closure of all sutures except in the fontanel region. The child started now having epileptical fits, which were controlled with phenobarbital. The child was subjected to psychiatric examination at 6 months age and this disclosed an intellectual level of 3½ months age. At 8 months age the head circumference had increased to 41 cm and the head was still more deformed, resembling the picture of a total craniosynostosis. The ventriculo-venous shunt was now removed and a new ventriculography was done, showing that the dilatation of the ventricles had diminished. Twenty four hours after the removal of the ventriculo-venous shunt the child went ill with bradycardia, mental deterioration and extension rigidity. It was decided that these symptoms were due to increasing intracranial pressure and a new ventriculo-venous shunt was inserted and at the same session, the right coronal suture, the right lambdoid suture and the sagittal suture were removed. During this operation it was found that the skull bones had grown together mostly with bony union but partly united by dense fibrous tissue. The postoperative course was uneventful, but later on the child developed periods of high fever, blood cultures were negative and no positive diagnosis could be established until the child died 11 months old in a purulent peritonitis, probably due to a persistent uraemia.



Fig. 1 and *b* Postoperative cranial deformity of the scaphocephalic type.
Fig. 2 and *b* Postoperat picture showing the features of total raniosynostosis.

Two of the cases with myelocele developed clinical and roentgenological signs of scaphocephaly within a very short time after the shunting procedure (Fig. 1*a* and *b*). The case of aqueductal stenosis showed very rapid collapse of the cranium with development of generalized cranio-synostosis (Fig *a* and *b*)

An interesting additional feature is the clearly visible increase in thickness of the









Fig. 3. (a) Preoperat X-ray of case in Fig 1 and b (b) Postoperati X-ray showing the scaphocephalic deformity and premature closure of the sagittal suture

Fig. 4. ( ) Preoperative X-ray of case in Fig 2 and b (b) Postoperati X ray showing premature suture closure and thickening of the cranial bones.

skull bones (Figs. 3*a* and *b* and 4*a* and *b*). The bones are much thicker than those of a normal child of the same age.

The scaphocephalic children have been treated conservatively

The case with total synostosis had its shunt removed but within 6 hours the child developed alarming signs of increased intracranial pressure and became acutely ill. This necessitated emergency reinsertation of a new shunt, this time using a medium pressure type In the same session a rightsided subtemporal decompression and splitting of the coronal, sagittal and lamboidal suture was done. Complete bony union of the sagittal suture was present. The coronal suture showed no synostosis but there was physiologically complete closure due to thick fibrous tissue interposed. Following operation the child's cranial features reverted to normal but unfortunately she succumbed three months later to purulent peritonitis.

Summary

Premature closure of the cranial sutures as complication to hydrocephalus surgery is previously only briefly mentioned in the literature An interesting feature is the abnormal thickening of the cranial bones, observed in the three cases presented. This might have something to do with the pressure that the normally growing brain probably exerts upon the bones during the development of the cranium.

The use of low pressure valves has now been discarded when operating hydrocephalus in our clinic.

The surgical correction of the secondary premature closure is probably more complicated than otherwise in these cases.

References

1. Dawson, B H.: Paper read at The Society for Research into Hydrocephalus and Spina Bifida, York, 1964.
Emery J L.: Paper read at The Society for Research into Hydrocephalus and Spina Bifida, Cambridge, 1963.
3. Matson D D.: Personal communication. 1964.
4. Pappas, G.: Cited by L. Strenger
5. Scarff J E.: Treatment of hydrocephalus an histological and critical review of methods and results. *J Neurol Neurosurg Psychiat*, 26 1, 1963.
6. Strenger, L. Complications of ventriculovenous shunts. *J Neurosurg* 20 219, 1963.
7 Yashon D. and Sugar, O Today problem in hydrocephalus. *Arch Dis Childh*, 39 58 1964.

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Renal Function and Blood Volume in Newborn Infant Related to Placental Transfusion

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During the past three decades, the renal function of the newborn infants have been extensively investigated and reviewed. Anatomically the glomeruli of the term neonates were shown to have some fetal characteristics [10 .1] and based on the limited number of clearance and challenge data, the kidneys of the newborn infants were often thought to be anatomically and physiologically immature [1 2 13 .4, 20]. Few of these studies were performed in the early hours of life to evaluate the immediate effects of perinatal events such as amount of placenta transfusion.

Recent works on the physiologic consequences of placental transfusion in the newborn infants have shown that blood volume [31, 20] hematocrits [16], central venous [12] atrial [3] and systemic arterial pressures [15] were significantly lower in infants given small amount of placental transfusion at birth by early cord clamping. Such hemodynamic and fluid volume differences could influence the immediate postnatal renal functional adaptation. This report attempts to correlate the glomerular and tubular functions of the newborn infants during the first 12 hours of life in relation to varied amount of placental transfusion by simultaneous measurement of urine flow Inulin and Para-aminohippurate clearances, filtration fraction, electrolyte excretions, and blood volumes. Similar studies were done in the older age group for comparison.

Material and Methods

Sixty nine female term newborn infants born vaginally at Södra Barnbördshuset, Stockholm, Sweden, to healthy mothers after 38 to 42 weeks of uncomplicated pregnancy were studied. Duration of labor ranged from 1 to 42 hours; fifty two mothers received intermittent inhalation of nitrous oxide ½ to 10 hours prior to delivery but none had premedications. All infants were in good condition t birth.

The infants were divided into two groups.

(1) Early clamped group (22 infants). The umbilical cords of these infants were clamped within 5 seconds after birth (delivery of the infant buttocks was considered as the time of birth). N attempt was made to determine the time relationship between onset of first breath and cord clamping.

(2) Late clamped group (47 infants). The cords of these infants were clamped after the arterial pulsations of the cord had stopped. No cord stripping was done.

All infants were delivered into the table

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where the mothers were lying and were about 10 cm below the mother's introitus.

Renal functions and blood volume studies were done in two different age gro ps; 1 to 12 hours and 2 to 5 days. All infants were breast fed starting at 12 hours of age. Each infant was studied only once.

The experiments were conducted in the laboratory where the room temperature was maintained at 24 to 26°C A control blood sample was obtained from a scalp vein. Thro gh the same scalp vein a primo dose of 10 % Inulin (20 mg/kg body weight) and 20 % Para-aminohippurate (Sodium p-aminoh ppurate, Merck, Sharpe and Dohme (7 mg/kg b.w) were injected w thin 1 to 2 minutes followed by an intravenous infusion of maintainance dose of Inulin (20 mg/min/1 73 M^2) and PAH (8 mg/min/1 73 M^2) mixed in a fluid mixture contai ing 1 part normal saline and 5 part 5 glucose in water running at 0.5 to 0.0 ml/mm., giving pproximately 10 ml/kg/hour Each infant received about 40 to 50 ml of the infusion fluid during the entire clearance period The plasma PAH nd Inulin equilibration was achieved at 25 to 30 minutes after the prime dose

Twenty minutes after the prime dose in jection 0 1 to 0.2 microcuries of I tagged serum albumin was injected into the scalp vein and flushed with 2 ml of saline through a three-way stopped cock for blood volume determinations. Care was taken to a oid leakage, r subcutaneous infiltration of the dose. F ve minutes after the injection, post-injection blood sample was obtained from either femoral or antecubital veins. The volemetron counter [35] was used to mea sure the radioactivity of the dose syringe before and after the injection, and on the pre- and post-injection blood samples. The final reading was mechanically calculated and expressed as blood volume in ml on the basis of a simple dil tion formula. The observed blood volume is corrected to the total body hematocrit by multiplying with a factor: (1 − venous hematocrit)/(1 − 0.87 venous hematocrit) The plasma and red blood cell volumes were calculated from the corrected blood volume multiplied by the venous hematocrit and 0.87 [14] The error of the volemetron method for blood olume determination as reported from this labora tory using the same machine and method was 4.1 % [33]. One drop daily of Lugol solution was given to the I treated infant for three days.

After the blood volume study the urinary bladder was catheterized aseptically using a French r bber cathet r size no. 9 to 11 with additional holes made on the tip The catheterization usually in ol ed minimal crying and excitement. However to avoid the effect f crying and pain on the renal functions [34], a minimum of 20 minutes were allowed between blood sampling, catheterization and the start of urine collection. No further venipuncture was done until the end of clearance period. During the collection period the infants were usually in a quiet state Complete emptying of the bladder was accomplished by flushing with 3 ml of sterile water followed by 3 ml of air No leakage was allowed around the catheter Depending on the amount f urine flow the duration of each collection period ranged from 5 to 20 minutes, and 2 to 4 such periods were done. The total urine olume (urine plus water for flushing) was measured on a 5 ml syringe calibrated to 0.2 ml divi sion and the actual urine volume and dilu tion calculated. Mean actual urine volume for each period was 1 2 ml. For plasma Inulin and PAH, sodium, potassium, and chlorid concentration, a plasma blank, 30 minute post primo dose and an end point blood samples were used for analysis. Plasma and urinary Inulin was measured by a direct resorcinol method without alkali treatment [23], PAH by the method of Goldring and Chasis [11] sodium and potassium by the flame photometer (Eppendorf) using Lithium standard, chloride by a microtitration method [25], and enous hematocrit (using preinfusion blood sample) by microcapillary tube technic [9] without correction for trapped plasma. All determina tions were done in d plicate and some in triplicate.

The effectiveness of antibiotics in the newborn infant for prophylaxis against infection is yet to be clarified [18]. None were used in our infants. One to 2 days after each experiment, clean voided urine samples were collected for bacteriuria screening using Triphenyl tetrazolium chloride (T T C.) test (Uroscreen, Pfizer), [28]. Three of the 60 infants tested revealed a positive T T.C. test, but catheterized samples subsequently obtained, showed negative results and were sterile bacteriologically. All infants were observed closely during their nursery stay: none developed complications and were discharged well on the 6th to the 10th day of life.

Calculations

Inulin and PAH clearances were calculated by the conventional formula of C = UV/P where C is the clearance in ml/min, U = the urinary concentration in mg/ml, V = urine volume in ml/min and P = plasma concentration in mg/ml. The plasma concentration of each period was derived from graph constructed from the pre- and post urine collection plasma concentration plotted against time, with correction made for the reading in the plasma blank. The precise plasma concentration on Inulin and PAH appropriate for each collection period was further corrected by 6 minute time delay for the formed urine to arrive at the bladder as recommended by Smith [31]. During the first 12 hours of life, the age period of urine collection was designed as age of the respective clearance.

While most of the investigators in neonatal renal physiology have used Inulin clearance to represent glomerular filtration rate [30], PAH clearance were considered not truly representative of the effective renal plasma flow (ERPF) since recent data on 6 infants under 2 months of age were shown to have a PAH extraction ratio of 64.4 [5]. For this reason, we do not emphatically equate C_{PAH} to ERPF; nevertheless, we calculated the effective renal blood flow (ERBF) by the formula:

ERBF = C_{PAH}/(1 − venous hematocrit).

It should be emphasized that the term ERBF is used in this report with some reservation until the question of PAH extraction ratio in the newborn period is settled in the future with larger series of observations.

To correct for variation in surface areas in our subjects, the urine flow C_{PAH}, C_{In}, ERBF and electrolyte excretion, were corrected to 1.73 M^2. The surface area was calculated from the nomogram of Bendroy and Cocchini [27].

To calculate tubular sodium reabsorption, we used the formula: Tna = kPnaCf − UnaV [31], where Tna = tubular sodium reabsorption in mM/min, k is the Donnan factor for Sodium ion, Pna = plasma sodium concentration in mEq/ml, Cf = filtration rate in ml/min (or glomerular filtration in ml/min, the product of kPnaCf represents total filtered sodium load; plasma sodium was the average of pre- and post-collection plasma sodium concentration. For uniformity with other related data, the Na excretion and reabsorption were also corrected to 1.73 M².

Results

The experimental data of this study are listed in Tables 1a to 1d and further summarized in Tables 2 to 5.

Blood volume and its constituents (Table 2)

During the first 12 hours of life the early clamped infants had a significantly lower blood volume (77 ml/kg) than the late clamped infants (91 ml/kg) and the difference persisted until the 5th day of life (81 ml/kg vs 94 ml/kg).

The red cell volumes were also significantly lower in early clamped infants (33.7 to 33.8 ml/kg) than in the late clamped infants (48.5 to 48.9 ml/kg) during the first 5 days of life while no difference was observed in the plasma volumes between the two groups. Venous hematocrits were significantly lower in the early clamped

TABLE 1 *Renal clearance data electrolyte excretion, and Blood Volume in 69 newborn infants*

Case	Birth wt (g)	Study wt (g)	Length (cm)	Age (hr)	Urine Inulin, mg %	Plasma Inulin, mg %	Urine PAH, mg %	Plasma PAH, mg %	Urine flow	CIN	CPAH	ERBF	FF	Na	K	Cl	BV	RBCV	PV	HCT venous %	S. A. m²
									ml/min/1.73 m²					Electrolyte excretion (μM/min/1.73 m²)			(ml/kg)				
( )	*Late clamped group (age 1 12 hours)*																				
1	4000	4020	56	1	400	10.0	490	3.0	0.70	15.0	118	207	.13	14.5	6.0	86.4	83	46	40	67	[illegible]
2	[illegible]	3000	50	1	700	17.4	616	5.0	0.42	17.7	103	276	.17	18.6	10.9	21.7	—	—	—	60	[illegible]
3	4150	4120	52	1	240	22.4	276	2.4	0.54	10.0	61	145	.16	19.1	8.4	25.7	79	40	39	58	[illegible]
4	3700	3700	53	2	450	8.2	720	4.9	0.45	22.0	90	222	.24	—	—	—	85	44	36	62	[illegible]
5	4700	4700	53	2	630	12.0	320	1.4	0.60	27.0	134	373	.20	—	—	—	85	53	42	64	[illegible]
6	3310	3310	48	2	340	8.5	190	1.42	0.70	22.1	104	237	.21	—	—	—	84	48	36	66	[illegible]
7	[illegible]	[illegible]	52.5	2	500	15.0	840	4.9	0.56	24.7	97	240	.20	—	—	—	91	47	44	61	[illegible]
8	3500	3400	51	2	770	24.5	604	4.5	0.42	14.0	51	142	.27	18	2.6	14.7	—	—	—	66	[illegible]
9	3010	3010	48	3	543	10.6	560	2.85	0.40	24.0	103	258	.23	—	—	—	91	48	44	60	[illegible]
10	2970	2970	48	4	470	8.8	423	2.0	0.06	34.3	142	401	.24	—	—	—	91	50	41	65	[illegible]
11	3700	3760	51	5	800	9.4	324	3.1	0.41	32.0	83	243	.33	—	—	—	—	—	—	73	[illegible]
12	4070	4070	52.5	5	572	10.5	375	2.2	0.94	33.0	100	234	.33	—	—	—	87	56	33	56	[illegible]
13	3350	3350	49	5	600	11.5	530	2.71	0.62	43.3	111	[illegible]	.30	—	—	—	87	43	44	56	[illegible]
14	3510	3510	49	5	354	22.0	350	4.0	1.32	21.0	83	277	.23	—	—	—	84	53	34	58	[illegible]
15	3700	3700	50	6	304	17.0	341	4.9	1.14	24.0	81	183	.26	—	—	—	—	—	—	54	[illegible]
16	3950	3950	48.5	6	700	13.0	343	1.6	0.37	19.0	77	143	.25	13.0	6.0	10.4	93	50	43	66	[illegible]
17	3200	3200	48	7	510	7.0	[illegible]	2.4	0.45	51.0	122	330	.43	—	—	—	86	48	42	64	[illegible]
18	4450	4400	51	7	300	12.5	121	1.0	1.16	24.5	70	174	.46	—	—	—	83	44	40	63	[illegible]
19	4100	4100	54	7	430	12.0	414	3.0	1.00	34.0	124	302	.25	—	—	—	100	57	41	58	[illegible]
20	3170	3120	[illegible]	7	304	17.5	[illegible]	2.45	1.24	21.0	143	433	.16	16.3	22.3	46.3	84	50	34	64	[illegible]
21	[illegible]	[illegible]	49.5	8	300	15.0	820	6.0	0.90	21.0	96	300	.23	—	—	—	104	67	[illegible]	74	[illegible]
22	[illegible]	3300	49	8	482	13.0	540	3.3	0.48	24.3	73	170	.23	—	—	—	90	44	[illegible]	56	[illegible]
23	3490	3400	[illegible]	8	657	14.0	[illegible]	3.25	0.53	21.0	[illegible]	100	.30	—	—	—	—	—	—	65	[illegible]
24	3510	3440	51	9	350	9.3	437	2.93	0.74	43.0	115	333	.34	—	—	—	100	57	43	[illegible]	[illegible]
25	[illegible]	3300	50	12	504	10.8	427	3.6	0.73	85.0	44	257	.41	—	—	—	93	49	43	62	[illegible]
26	[illegible]	3400	51	12	565	6.0	[illegible]	2.7	0.72	31.0	43	160	.31	—	—	—	85	37	40	51	[illegible]
27	3500	3440	51	12	810	13.5	333	2.9	0.90	32.0	90	191	.26	—	—	—	80	41	46	63	[illegible]
28	3140	3140	47	12	493	17.0	302	4.8	0.84	24.0	[illegible]	166	.35	—	—	—	87	50	47	56	[illegible]
29	[illegible]	3500	51	12	305	11.0	[illegible]	4.06	0.64	23.0	70	207	.33	—	—	—	95	51	44	63	[illegible]
?	*Early clamped group (age 1 to 12 hours)*																				
1	3050	3060	50	1	620	19.0	100	1.0	0.43	14.2	30	68	.20	19.2	18.3	30.5	[illegible]	40	42	[illegible]	[illegible]
2	4220	4220	54	2	640	14.5	700	3.05	0.30	22.4	[illegible]	170	.36	—	—	—	73	36	37	54	[illegible]
3	3000	3000	48	3	[illegible]	12.0	[illegible]	1.82	0.30	19.5	113	233	.17	24.7	8.0	40.1	80	37	43	52	[illegible]
4	3310	341	51.3	3	125	23.8	925	3.0	0.30	14.0	90	200	.16	20.7	14.5	23.6	78	33	40	37	[illegible]
5	3000	4000	49.5	4	447	13.1	650	4.9	0.61	30.0	70	135	.26	21.9	13.3	10.6	77	37	[illegible]	40	[illegible]
6	3700	3700	52	5	300	7.0	190	3.0	0.61	25.2	30	80	.47	—	—	—	77	34	43	51	[illegible]
7	3470	3470	[illegible]	5	470	7.0	343	2.6	0.48	23.0	83	110	.47	—	—	—	79	33	46	40	[illegible]
8	3000	3000	51.5	5	730	20.4	573	4.22	0.50	19.0	73	158	.25	—	—	—	74	33	41	51	[illegible]
9	4070	4070	53	5	545	10.0	453	2.1	0.22	13.0	0	153	.17	13.0	4.1	13.3	70	25	42	54	[illegible]
10	3300	3300	49	6	573	14.5	[illegible]	2.1	0.42	17.0	67	130	.23	20.4	14.4	84.0	70	37	43	53	[illegible]
11	3330	3000	50	7	619	16.0	400	4.13	0.93	22.5	84	110	.38	—	—	—	72	[illegible]	37	41	[illegible]
12	2930	2950	4[illegible]	12	430	14.0	617	4.5	0.74	22.6	78	130	.37	—	—	—	61	[illegible]	53	41	[illegible]
13	3030	3030	51	12	730	16.0	[illegible]	2.0	0.85	26.5	71	143	.37	—	—	—	78	35	43	51	[illegible]
14	3100	3100	40	12	[illegible]	16.6	303	4.4	0.67	18.0	60	118	.37	—	—	—	72	30	44	40	[illegible]

CIN Inulin clearance CPAH PAH clearance, ERBF Effective renal blood flow, FF Filtration fraction, Na sodium, K potassium, Cl Chloride, BV blood volume, RBCV red blood cell volume, PV = plasma, HCT hematocrit, S. A. surface area.

TABLE 1 (*continued*)

	Birth wt (g)	Study wt (g)	Length (cm)	Age (days)	Urine Inulin, mg %	Plasma Inulin, mg %	Urine PAH, mg %	Plasma PAH, mg %	Urine flow (ml/min/1.73 m²)	C_{IN} (ml/min/1.73 m²)	C_{PAH} (ml/min/1.73 m²)	ERBF (ml/min/1.73 m²)	FF	Electrolyte excretion Na (μM/min/1.73 m²)	K (μM/min/1.73 m²)	Cl (μM/min/1.73 m²)	BV (ml/kg)	RBV (ml/kg)	PV (ml/kg)	HCT venous %	S.A. m²
(c)	*Late clamped group (age 2–5 days)*																				
1	3370	3180	50	2	230	15.0	144	1.9	2.84	43.6	210	424	.20	—	—	—	118	53	65	50	.22
2	3330	3120	49.5	2	480	13.0	400	2.1	0.80	32.0	115	296	.28	—	—	—	82	50	42	62	.22
3	3870	3830	48	2	560	13.3	240	1.9	1.34	56.7	163	506	.32	—	—	—	96	56	40	60	.20
4	3600	3700	50	2	610	12.28	300	2.60	1.81	78.0	187	430	.40	28.5	9.0	31.4	83	50	50	60	.23
5	3650	3630	51	2	605	17.3	700	5.75	0.80	31.6	94	238	.34	30.5	22.0	36.0	—	—	—	60	.24
6	3300	3140	50	2	180	10.8	182	4.05	1.34	13.0	48	147	.24	33.0	11.4	13.6	90	40	37	65	.23
7	3000	3030	49.5	2	310	15.0	280	2.8	0.82	17.0	79	196	.22	8.4	10.4	13.4	82	4	38	62	.21
8	4250	4000	51	2	300	16.0	250	2.4	1.15	24.0	110	371	.20	12.4	20.0	15.8	91	54	39	65	.24
9	3000	3570	50	3	585	10.5	340	1.8	0.63	35.7	95	190	.38	—	—	—	93	40	58	50	.21
10	3050	3000	51	3	640	11.0	270	1.36	0.64	37.3	140	296	.27	—	—	—	94	44	60	53	.23
11	3000	3360	52	3	110	11.1	570	2.5	5.30	54.0	182	635	.30	—	—	—	90	40	60	57	.23
12	3640	3400	52	3	315	16.0	650	2.11	1.71	53.0	283	787	.19	—	—	—	98	52	43	60	.24
13	2800	2740	49.5	3	555	2.13	540	3.30	0.86	22.3	74	193	.30	—	—	—	105	51	54	62	.21
14	4100	4000	54	3	730	14.0	416	1.30	0.61	30.0	225	400	.13	—	—	—	91	44	47	65	.26
15	3000	3450	48	3	300	20.7	112	2.	1.00	23.5	58	144	.40	11.8	19.0	29.2	82	43	38	60	.22
16	3340	3090	50	3	864	30.2	400	2.77	0.97	18.0	173	400	.10	20.8	16.8	13.8	80	47	35	63	.23
17	3030	3300	50.5	4	504	12.3	180	2.55	1.10	47.5	80	250	.50	—	—	—	104	63	48	61	.23
18	4000	3870	50	5	405	7.3	124	1.10	1.06	67.0	114	5.3	.30	—	—	—	—	—	—	60	.24
(d)	*Early clamped group (age 2–5 days)*																				
1	3350	3300	50.5	2	403	14.7	128	1.83	1.13	31.5	97	164	.33	—	—	—	62	30	32	41	.22
2	3430	3400	50	2	320	10.5	910	3.00	0.00	23.3	206	416	.14	—	—	—	78	34	44	58	.23
3	4250	4000	54	3	—	—	—	—	0.27	—	—	—	—	10.4	10.4	32.0	—	—	—	50	.25
4	3340	3370	52	3	664	11.6	400	2.63	0.06	34.2	124	313	.30	17.0	14.7	30.1	77	36	46	67	.23
5	3600	3360	47	3	330	12.9	118	2.90	2.01	44.8	107	210	.42	—	—	—	78	36	44	51	.22
6	3340	3390	49	4	—	—	—	—	0.62	—	—	—	—	34.0	4.8	133.4	—	—	—	46	.22
7	4436	4140	53	4	111	13.5	40	1.45	4.60	40.0	152	236	.33	43.0	7.6	183.1	86	36	54	43	.23
8	3400	3400	54	5	40	21.5	304	2.40	0.00	17.0	106	183	.10	18.4	13.4	34.4	83	33	63	43	.23

TABLE 2 *Blood volume, red cell volume, plasma volume and venous hematocrits in 69 newborn infants with early and late cord clamping from 1 hour to 5th day of life.*

Values are expressed in means ± standard of the means.

Age	1–12 hours			2–5 days		
Group	Early clamped	Late clamped	p	Early clamped	Late clamped	p
Blood volume (ml/kg)	77.0* ±0.99	91.0* ±1.21	<.001	81.0* ±1.39	94.0* ±2.40	<.001
Plasma volume (ml/kg)	43.0 ±1.00	42.0 +0.98	>.05	49.0 ±1.76	48.1 ±1.78	>.05
Red cell volume (ml/kg)	33.7* ±1.0	48.5 ±1.21	<.001	33.9* ±1.18	48.9* ±1.33	<.001
Venous hematocrits (%)	50.0* ±1.36	62.0* ±1.13	<.001	48.0* ±2.2	61.0* ±1.22	<.001
Number of infants	14	20[a]		8[b]	18[b]	

Differences are significant with all *p* values of < 0.001.

[a] Five and [b] two of the infants did not have blood, plasma, and red cell volume determinations.

TABLE 3 *Urine flow glomerular filtration rate, PAH clearance, effective renal blood flow, and filtration fraction in 69 newborn infants with early and late cord clamping during the first 5 days of life.*

Values are expressed in means ± standard error of the means.

Age	1 12 hours			2–5 days		
Group	Early clamped	Late clamped	p	Early clamped	Late clamped	p
Urine flow (ml/min/1 73 M^2)	0.51 ±0.040	0.75 ±0.039	< 001	1.75 ±0.57	1 44 ±0.25	>.05
Glomerular filtration rate (ml/min/1.73 M^2)	20.3 ±1.2	28.6* ±1 9	< 001	32.0 ±2.6	37.5 ±4.1	>.05
PAH clearance (ml/kg/1.73 M^2)	71.0* ±2.8	96.0* ±4.4	< 001	129.0 ±15.0	136.0 ±14.0	>.05
Effective renal blood flow (ml/kg/1 73 M^2)	142.0* ±10.9	259.0* ±15.0	< 001	234.0 ±23.0	250.0 ±25.5	> 05
Filtration fraction	0.31 ±0.03	0.31 ±0.02	>.05	0.27 ±0.03	0.30 ±0.03	>.05
Number of infants	14	29		8*	18	

Two infants had no clearance measurements.

infants (48 to 49% *vs* 61 to 62%) from birth to 5th day of age.

Urine flow and renal clearances (Table 3 Fig. 1)

In spite of wide variations in their values *during the first 12 hours of life* the early clamped infants had a significantly lower urine flow (0.51 *vs.* 0 75 ml/min/1 73 M^2), Inulin clearance (20.3 *vs* 28.6 ml/min/1.73 M^2) PAH clearance (71.0 *vs* 96 ml/min/1 73 M^2) and effective renal blood flow (142 *vs* 259 ml/min/1 73 M^2) than the late clamped infants. All p values were < 0.001 The filtration fractions calculated from C_{IN} divided by C_{PAH} were the same in both groups Significant increment in urine flow and renal clearances were observed in both groups of infants when they became older (2–5 days old). The magnitude of increment is relatively more in the early clamped infants since their clearances were lower in the early hours of life and became comparable with the values of the late clamped infants during the 2nd to the 5th day of age

Correlation between blood volume and its components with renal clearances and urine flow (Fig. 2 to 4)

Grouping early and late clamped infants together Fig 2 shows positive correlation between glomerular filtration rates (GFR) and blood volume and between urine flow and blood and red cell volumes. Correlation coefficients for GFR *vs* plasma volume red cell volume and hematocrits and for urine flow *vs* plasma volume and hematocrits were not significant.

PAH clearance were directly related to blood volume, venous hematocrit and red cell volume (Fig. 3)

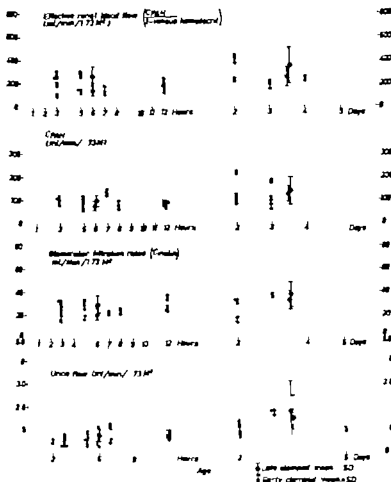


Fig. 1 Urine flow glomerular filtration rates, effective renal plasma and blood flows in 60 infants with early and late cord clamping during the first 5 days of life.

The ERBF shows the same correlation as C_{PAH} (Fig 4) moreover the correlation was also present with the late clamped infants alone (slopes represented by dotted lines) ERBF is indirecty related to plasma volume.

Urinary electrolyte excretion

In Table 4, the urinary sodium (Na) potassium (K), and chloride (Cl) excretion in 24 infants were summarized. A higher Na excretion in early clamped infants during the first 12 hours of life was observed when compared with the late clamped infants ($p = 0.05 - 0.01$).

In older infants (? to 5 days old) the same trend seems to be present, but the difference was not statistically significant.

In Table 5 the renal Na transport in 12 newborn infants 1 to 7 hours of life were summarized. The late clamped infants

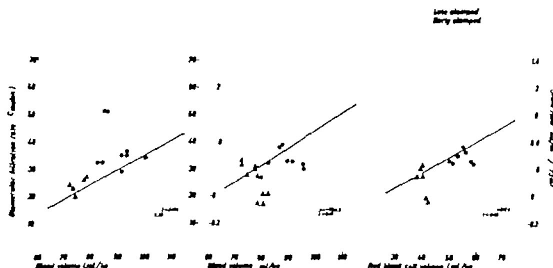


Fig. 1. Correlation between glomerular filtration rates and blood volume, and between urine flow and blood and red cell volume during the first 12 hours of age in 38 infants with early and late clamping of the cords.

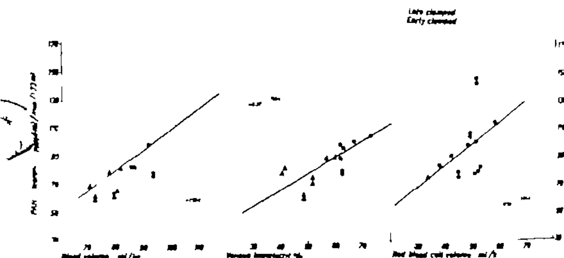


Fig. 2. Correlation between PAH clearance and blood volume, venous hematocrits and red cell volume in 38 infants with early and late cord clamping during the first 12 hours of life.

filtered slightly higher Na load than the early clamped infants although the difference was not statistically significant. However the late clamped infants excreted significantly less amount of filtered Na load, indicating greater Na reabsorption in the tubular system as shown by the significantly higher percentage of tubular Na reabsorption relative to total filtered Na load in the late clamped group ($p < .05$) In older infants the filtered Na load, urinary Na excretion and tubular Na

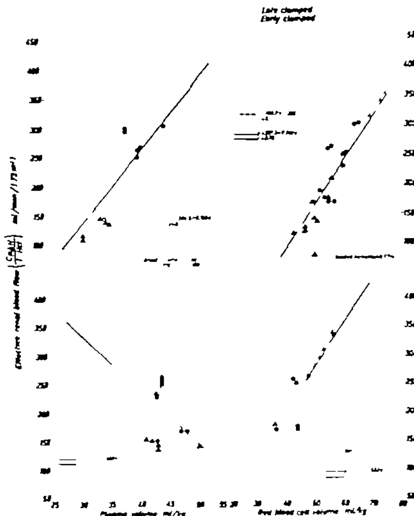


Fig. 4. Correlation between effective renal blood flow and blood volume, plasma volume, venous hematocrit and red cell volume in 33 infants with early and late cord clamping during the first 12 hours of life. Solid lines represent the slopes of regression lines for both early and late clamped infants while the dotted regression lines represent the late clamped infants alone.

reabsorption were the same in both groups of infants.

Discussion

Our blood volume data supplement the findings of previous investigators indicating that infants given large amount of placental transfusion by late cord clamping at birth had a larger blood and red cell volume and venous hematocrits than the early clamped infants [8, 16, 20, 22 26, 29 33].

It is known that one of the major factors regulating glomerular filtration rates (GFR) is the glomerular capillary blood

TABLE 4. *Urinary electrolyte excretion in 24 newborn infants with early and late cord clamping during the first five days of life.*

Means ± standard error of the means.

Group	1 12 hours		2–5 days	
Group	*Early clamped*	*Late clamped*	*Early clamped*	*Late clamped*
Sodium (μM/min/1 73 M^2)	20.0 ±1.50	14.9* ±1.52	26.5 ±7.50	17.9 ±2.10
Potassium (μM/min/1.73 M^2)	11.5 ±2.70	9.2 ±2.98	10.6 ±1 70	16.1 ±2.50
Chloride (μM/min/1.73 M^2)	32.0 ±6.90	27.2 ±4 07	64.4 ±19.6	22.9 ±6.0
Number of infants	6	6	5	7

Difference of urinary sodium excretion between early and late clamped infants is significant ($p = 0.05 - 0.01$).

TABLE 5 *Renal sodium transport in 12 newborn infants with early and late cord clamping during first 7 hours of life.*

Pna = Plasma sodium; kPnaCf = Total filtered sodium load or product of Donnan factor for Na Pna Cf; Cf = glomerular filtration rate (C_{in}); UnaV = Urinary sodium excretion; Tna Total tubular sodium reabsorption.

Case no.	Age (hr)	Pna (mEq/ml)	Cf (ml/min/ 1.73 M^2)	*kPnaCf*	*UnaV*	Tna	Tna % of filtered load
					M/min/1 73 M^2		
Late clamped group							
1	1	0.128	15.0	1.8048	0.0145	1 7355	99.93
2	3	0.150	17 7	2.4957	0.0156	2.4807	99 40
3	2	0.128	13.0	1.6048	0.0191	1 7858	98.95
8	3	0.128	14.0	1.8645	0.0100	1.8747	99 42
16	6	0.126	19.0	2.2504	0.0134	2.2370	99.42
20	7	0.122	21 1	2.6181	0.0162	2.6016	99.37
M		0.123	17 0	2.1097	0.0149	2.0948	99.33
s.d.		±0.009	±2.7	±0.3450	±0.0030	±0.3990	±0 236
Early clamped group							
1	1	0.128	14.2	1 7085	0.0193	1 6893	98.88
3	2	0.129	19.8	2.3462	0.0247	2.3213	98.94
4	2	0.126	14.6	1.7293	0.0203	1 7090	98.83
5	4	0.132	20.9	2.6933	0.0219	2.8714	99 19
9	6	0.128	12.0	1.4438	0.0129	1 4309	09.10
10	5	0.120	17 0	2.0774	0.0204	2.0570	99.00
M		0.129	16.3	1.9630	0.0200	1.9430	98.97
s.d.		±0 0024	±3.3	±0.4330	±0.00347	±0.4310	±0.124
Significance of difference		—	—	—	$p <$ 05	—	$p <$ 05

pressure and the latter is normally the same as the aortic pressure [4, 32]. Assuming that other factors influencing GFR as permeability and areas of the glomerular membrane, intracapsular pressure and colloid osmotic pressure of the plasma protein were the same in early and late clamped infants, the higher GFR in the late clamped infants is probably due to a higher glomerular capillary pressure since it has been shown that these infants had a significantly higher systolic blood pressure during the first 2 hours of life than the early clamped infants [15]. Furthermore, positive correlation has been shown between systolic blood pressure and hematocrits and between hematocrits and blood volume [14, 33]. These evidences seem to point out a direct and multirelationship between blood volume, arterial blood pressure and glomerular filtration rates.

The higher urine flow observed in the late clamped infants during the first 12 hours of life is apparently a result of greater GFR. This finding is consistent with Barnett's observation of direct correlation between GFR and urine flow in young infants [2].

The exact mechanism for the higher PAH clearance and (if the PAH extraction ratio were the same in both groups) effective renal plasma and blood flow in the late clamped infants, is less obvious. The most likely explanation may still be the fact that they had higher blood volume than the early clamped infants. The direct correlation found between PAH clearance and blood volume further supports this hypothesis.

Newborn infants as young as first 7 hours of age reabsorbed almost 100% of filtered sodium load, attesting the functional integrity of tubular Na reabsorption process.

The observed greater percentage of tubular Na reabsorption and higher urine output in the late clamped infants when compared with the early clamped infants, represent the renal aspect of body fluid regulation during the first few hours of life. In these infants, a process of fluid shift involving capillary fluid transudation from the vascular to extravascular spaces occurred during the first 4–6 hours of age [7, 16, 33]. This movement of fluid necessitates a compensatory redistribution of water and electrolytes with retention of Na ion in the extravascular compartments, which accounts for the greater tubular reabsorption, and less urinary excretion of this ion. It is interesting that a higher urinary output was observed in the presence of low sodium excretion. It is evident that in the late clamped infants, body fluid and electrolyte redistribution and glomerular and tubular functional adaptations achieved the necessary adjustment imposed by the vascular distension and transudation resulting from placental transfusion at birth. These changes do not occur in the early clamped infants since little or no fluid shift occurred. This is another striking example of how body compositional alteration in the early neonatal period could affect urine composition and concentration [19].

Our data on urinary Na excretion were the opposite of those obtained by Cort and Pribylova [6] who found a significantly higher Na excretion in the late clamped infants. Since the designs of our experiments were somewhat different no comparative explanations could be drawn.

Antidiuretic hormones (ADH) could

play a role in the regulation of water and electrolyte metabolism. However our study has no direct evidence to demon strate this hypothesis.

Continuous intravenous infusion of rela tively hypotonic fluid during the experiment could conceivably affect our data on water and electrolyte metabolism and to some extent the clearances. However since the amount of fluid and electrolytes given were carefully controlled, the observed differences between early and late clamped infants would probably not be a function of fluid intake

Repeated measurements of urine flow GFR PAH clearance and ERBF on the 2nd to 5th day of life showed no difference between the early and late clamped infants in spite of persistent discrepancy in blood volume (Tables 2 and 3) The early clamped infants achieved effective renal functional adaptation through mechanisms other than blood volume changes. It is interesting to note that a very similar pattern of changes in relation to age were also observed in systemic arterial pressures, central venous pressures, and peripheral temperature in early clamped infants [12 15 17] These investigations showed that the early clamped infants had lower arterial blood pressures, central venous pressures and peripheral skin temperatures during the first few hours of life when compared with the late clamped infants and when they became older the differences were no longer observed. Blood volume probably played a more imminent role in cardiovascular renal and thermogenic adaptation during the first hours of life than in the older infants.

Summary and Conclusion

Inulin and PAH clearances, urine flow filtration fraction, electrolyte excretion and blood volume studies were performed on 43 term vaginally delivered female normal infants during the first 12 hours of life and 26 during the 2nd to the 5th day of age The umbilical cords of 22 infants were clamped within 5 seconds after birth and in 47 infants, the cords were clamped after their arterial pulsation had stopped.

Compared with the late clamped infants, the blood volume, red cell volume and venous hematocrit were lower in the early clamped infants during the first five days of life The urine flow glomerular filtration rate (GFR) PAH clearance and effective renal blood flow were also lower in the early clamped infants during the first 12 hours of life presumably due to the lower blood volume and blood pressures. At 2–5 days of age the urine flow GFR, PAH clearance and effective renal blood flow were the same in both groups of infants in spite of persistent difference in blood volume The early clamped infants apparently achieved renal adaptation through means other than blood volume compensation.

Data on electrolyte metabolism suggest that the late clamped infants filtered and reabsorbed larger amount of sodium than the early clamped infants during the first 6 hours of life accompanied by a greater urine flow These findings were appropriately achieved by the kidney in the process of body fluid regulation in response to the vascular distension and fluid transudation resulting from placental blood transfusion at birth.

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References

1. Barnett, H. L.: Kidney function in young infants. *Pediatrics*, 5: 171 1950.
2. Barnett H. L. and Vesterdal, J. The physiologic and clinical significance of immaturity of kidney function in young infants. *J Pediat*, 42 99 1953.
3. Burnard, E. D and James, L. S.: Atrial pressures and cardiac size in the newborn infant. *J Pediat*, 62 815, 1963.
4. Best, C. H. and Taylor, N B *The Physiologic Basis of Medical Practice*. Williams and Wilkins Co., Baltimore, 7th ed., 1961, p. 537
5. Calcagno, P. L. and Rubin, M. I.: Renal extraction of para-aminohippurate in infants and children. *J Clin Invest*, 42 1632. 1963.
6. Cort, R. L. and Pribylova, H.: Placental transfusion and fluid metabolism on the first day of life. *Arch Dis Child*, 39 363, 1964.
7. Gairdner, D Marks, J Roscoe, J D and Brettell, R. O The fluid shift from the vascular compartment immediately after birth. *Arch Dis Child*, 33. 489 1958.
8. Gunther, M.: The transfer of blood between baby and placenta in the minutes after birth. *Lancet*, 1 1277 1957
9. Guest G. M. and Siler, C. E.: Centrifuge method for determination of volume of cells in blood. *J Lab & Clin Med* 19 757 1934.
10. Gruenwald, P and Popper, H.: Histogenesis and physiology of the renal glomerulus in early postnatal life Histological examination. *J Urol*, 43 452, 1940.
11. Goldring, W and Chasis, H.: *Hypertension and Hypertensive Disease* (appendix D Methods). The Commonwealth Fund, New York, N Y 1944
12. Jegier, W Blankenship W and Lind, J Venous pressure in the first hour of life and its relationship to placental transfusion. *Acta Paediat Scand*, 52 485, 1963.
13. McCance, R. A.: Renal physiology in infancy *Am J Med* 9 229 1950
14. Mollison, P L., Veall, N and Cutbush, M.: Red cell and plasma volume in newborn infants. *Arch Dis Child*. 25 242, 1950.
15. Oh, W Lind J and Gessner, I H Circulatory and respiratory adaptation to early and late cord clamping in the newborn infants. *Acta Paediat Scand*. In press.
16. Oh, W and Lind J Venous and capillary haematocrit in the newborn infant and placental transfusion. *Acta Paediat Scand*. In press.
17. — Body temperature in the newborn infants and placental transfusion. In manuscript.
18. Oh, W Keller, R., Klein R. L and Kuttstadter, R. H.: Antibiotics in infants for premature rupture of membranes. *Am J Dis Child*, 108. 149 1964.
19. Oh, W Stewart L., Barnes, G R. and Metcoff J Body composition and renal adaptation in the newborn rat. *Biol Neonat*, 8 107 1965.
20. Oh, W Blankenship W and Lind J Further studies of blood volume in newborn infants during the first 2 hours of life related to placental transfusion. In manuscript.
21. Potter, E. L. and Thierstein S. T Glomerular development in kidney as index of fetal immaturity *J Pediat*, 22 695, 1943.
22. Redmond A., Isana, S. and Ingall, D Relation of onset of respiration to placental transfusion. *Lancet* 1 283, 1965.
23. Roe, J H., Epstein L H. and Goldstein N P A photometric method for the determination of Inulin in plasma and urine. *J Biol Chem*, 178 839, 1949.
24. Rubin M. L., Bruck, E. and Rapoport M.: Maturation of renal function in childhood. *J Clin Invest*, 28. 1144, 1949.
25. Schales, O and Schales, S. S.: Simple and accurate method for determination of chloride in biological fluids. *J Biol Chem*, 140 879, 1941
26. Secher, O and Karlberg, P Placental blood transfusion. *Lancet*, 1 1203, 1962.
27. Sendroy J J and Cecchini, L. P Determination of human body surface area from height and weight. *J Appl Physiol*, 7 1 1954.
28. Simmons, N. A. and Williams, J D A simple test for significant bacteriuria. *Lancet* 1 1377 1962.
29. Sisson T. R. C. and Whalen, L. E.: The blood volume of infants. III. Alterations in the first hours after birth. *J Pediat*, 56 43, 1960.

30. Smith, C. A.: *The Physiology of the Newborn Infant.* Blackwell's Scientific Publication, Oxford, 3rd ed., 1959.
31. Smith, H. W. *Principles of Renal Physiology.* Oxford University Press, New York, N.Y. 1956, p. 199.
32. Swann, H. G., Moore, V. and Montgomery, A. V.: Influence of arterial pressure on intrarenal pressure. *Am J Physiol, 168* 637 1952.
33. Usher, R., Shephard, M. and Lind, J.: The blood volume of the newborn infant and placental transfusion. *Acta Paediat Scand, 52* 497 1963.
34. Vesterdal, J. and Tudvad, F.: Studies on the kidney function in premature and full term infants by estimation of the Inulin and Para-aminohippurate clearances. *Acta Paediat Scand, 37* 429 1949.
35. Williams, J. A. and Fine, J. Measurement of blood volume with a new apparatus. *New Engl J Med, 264.* 842, 1961.

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Urinary Excretion of I^{131} Diodrast Injected Intraventricularly in Communicating Hydrocephalus and Aqueduct Stenosis

by GUNNAR GROTTE, IRÉNE SJÖGREN and HÅKAN SJÖGREN

The place and mode of absorption of the cerebrospinal fluid (C.S.F.) is still not quite understood. In most cases of expansive hydrocephalus, some kind of interference with this absorption occurs [4, 5].

In 1961, Pappenheimer and his co-workers described the first evidence that the transfer of certain C.S.F. components from the C.S.F. pathways in the central nervous system into the vascular system may be due to an active transport; their experiments on goats showed how Diodrast and other substances rapidly disappear from the C.S.F. by an "active mechanism of transport situated in and around the fourth ventricle [13]. As in other mechanisms of active transport, there is a certain limit of transportation rate [13, 14]. There is also a passive transport, including diffusion as well as absorption in bulk".

If these conditions were the same in human beings as in goats, they could be used for developing a test for diagnosing the patency or stenosis of the aqueduct of Sylvius. We therefore tested Pappenheimer's conclusions by injecting I^{131} Diodrast into the ventricular system of children with infantile expansive hydrocephalus. We found a difference in transport velocity when the cases with free communication through the aqueduct, the fourth ventricle and downwards were compared with those cases in which the C.S.F. transport was cut off because of a stenosis of the aqueduct.

Material

The clinical material consisted of nine infants, five boys and four girls (see Table 1) aged 2–14 weeks, suffering from untreated expansive hydrocephalus and attending the University Hospital, Uppsala. All the children showed pathological skull growth, bulging fontanel, diastasis of the sutures, stasis of the scalp veins, "sunset" phenomenon and dilated ventricles. In all cases the hydrocephalus diagnosis was confirmed by echo encephalography and air encephalography and, in one case, by post-mortem examination as well.

Four children had stenosis of the aqueduct: in two cases due to intra-uterine toxoplasmosis (cases no. 1 and 2) and in the two others due to congenital malformations of unknown origin (cases no. 3 and 4), of which one (case no. 3) already showed obvious hydrocephalus at birth. The increase of head circumference was rapid. Neither at lumbar air encephalography nor at ventri-

culography was there any passage of air through the aquoduct.

One child (case no. 5) with a lumbar myelomeningocele had a rather rapidly increasing hydrocephalus. At the ventriculography only small amounts of air passed through a malformed and partially obstructed aqueduct.

Two of the children (cases no. 6 and 7) showed communicating hydrocephalus. In one of these cases (case no. 7), there was a history of difficult delivery and perinatal brain haemorrhages, evidently leading to basal cistern block and hydrocephalus. In the other case (case no. 6) there was a lumbosacral myelomeningocele. The rate of skull growth was comparatively slow. At pneumo-encephalography the air passed with no difficulty through the aqueduct of Sylvius.

In cases no. 8 and 9 no air passed through the aqueduct at the neuroradiological investigation. However both children had myelomeningoceles (in which the difficulties of getting an air passage through the aqueduct, even if patent, are well known). The skull growth was comparatively slow indicating communicating hydrocephalus. In case no. 9 it was later verified at a post mortem examination that the aqueduct was normal.

All children excreted ordinary volumes of urine and had normal specific gravity of the urine. None had proteinuria or evidence of urinary infection.

After the Diodrast investigation all the children were shunt-operated by the Spitz-Holter method before they reached the age of four months and were not available for further studies of the patency of the aqueduct.

Method

Before the investigation, the infants were given Lugol's solution, one drop on two days and one drop the day before the investigation. The test substance used was I^{131} Diodrast from Abbot Laboratories, North Chicago, Ill. U.S.A. It was diluted to a suitable concentration by physiological NaCl solution. The total amount injected varied between 0.2 ml and 1 ml proportionally to the degree of the hydrocephalus, and was calculated never to exceed 2 mg % of Diodrast intraventricularly.

Urine was collected through a catheter in the urinary bladder every tenth minute for the first hour and every half-hour during the following 3–4 hours. In two cases all the urine was also collected in plastic bags for two and three days respectively after injection. Extra fluids were given by mouth to stimulate urine production during the test.

When calculating the concentration of the Diodrast and the maximum dose given to the infant the ventricular system was regarded as a sphere, the volume of which can be approximately calculated when the skull circumference and the thickness of the brain parenchyma are known [7]. The radioactivity of the I^{131}-Diodrast injected varied between 0.4 and 5 microcuries. One microcurie or less was found to be sufficient. The maximum radiation dose was obtained by adding the doses due to gamma and beta rays [8] given to the brain parenchyma in and surrounding the approximately spherical liquor compartment in the theoretical case in which no transport of I^{131}-Diodrast from the ventricles takes place. In this case the effective half time is equal to the half time of the I^{131} which is 8 days. In this extreme case 1 microcurie was calculated to give a dose of 200 millirad. In fact the effective half time was considerably lower especially in cases of communicating hydrocephalus, where the actual dose was less than 50 millirad.

TABLE 1 *Patient material and results.*

				Clinically					
Sex	Patient	Age in months	Head circumference in cm	General	Neurological symptoms	Increase in head circumference	Radiologically	Biological halftime of I^{131}	
♂	M. H. 631126	3	48	(1–3) intrauterine toxoplasmosa. Hydrocephalus at birth	+	(1–4) Rapid, mean value 1.5 cm/week	(1–4) Aqueduct stenosis	1 days	2 da
♀	C. G. 631225	3½	47		−			3 days	
♀	H. P 630220	1	44		+			10 day	
♀	C.S. 630302	1	30	Immaturity and/or asphyxia	+			2 day	
♂	M. G 630703	3½	51	Myelomeningocel	+	1 cm/week	Malformation with partial aqueduct stenosis	1½ day	
♂	R. K. 630406	1½	36	Myelomeningocele	+	(6 & 7) Moderate 0.8 cm/week	Communicating hydrocephalus	10 hours	<1 da
♂	K. P 630220	3	43	Perinatal damage	+			24 hours	
♀	K. G 631121	3½	42	(8 & 9) Myelomeningocele	−	Moderat	∖ passage of air through the aqueduct	8 hours	
♂	K. L. 630401	½	43		+			14 hours	

Dead from septicemia postoperatively ∖ aqueduct stenosis.

Repeated blood tests taken after the intraventricular injection of I^{131} Diodrast showed hardly measurable radio-activity demonstrating that the concentration of Diodrast in the blood was practically zero

All samples were measured in a well-type scintillation counter The efficiency of the counter was found to be about 50 per cent.

The radioactivity remaining in the body is determined as a function of time by successively subtracting the xcreted radioactivity from the injected radioactivity which is known In a semi-logarithmic diagram a straight line is usually obtained from 2 hours after injection and later and the slope of that line determines the biological half time

All children were doing as well after as before the puncture and there were no side effects.

Results

The results of the investigations are shown in Table I and Figs. 1 and 2 In two of the cases with communicating hydrocephalus (cases no 6 and 7) large concentrations of I^{131} were already found in the urine during the first 4 hours after injection, while the excretion in the urine

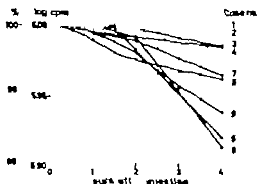


Fig. 1

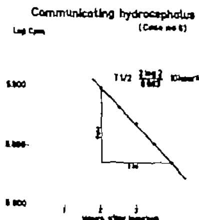


Fig. 2

Fig. 1. Logarithm of retained radioactivity normalized to 10^4 cpm injected, as function of time. Left hand axis gives the same radioactivity as a percentage. Retained radioactivity is calculated as the injected radioactivity minus the radioactivity excreted in the urine.

Fig. 2. Calculation of the biological half-time of I^{131} after intraventricular injection of Diodrast into a child with communicating hydrocephalus.

for the cases with stenosis of the aqueduct (cases no 1–4) was considerably delayed. The biological half-time for I^{131}-Diodrast was thus 10–24 hours in the cases no. 6 and 7 with communicating hydrocephalus compared with 2–12 days in the cases no. 1–4 with stenosis of the aqueduct. In the two cases in which the neuroradiological investigation showed no passage of air through the Sylvian aqueduct (cases no 8 and 9) but in which the case histories and the clinical pictures suggested communicating hydrocephalus rapid excretion of I^{131} Diodrast was obtained with half times of 8–14 hours, as in the cases with communicating hydrocephalus. One child (case no 9) died after the operation, apparently from sepsis; at the post-mortem examination no malformation of the aqueduct was found, nor were there any Arnold–Chiari malformations.

Case no. 5 is a borderline case, clinically radiologically and in relation to the biological half time of the I^{131} and is explained by a narrow and malformed aqueduct.

In the two cases (nos. 1 and 4), in which calculations were performed using the total amount of I^{131} excreted during 48 hours, we obtained identical half-times as when using the first 4 hours after injection.

Discussion

As regards the mechanism of C.S.F absorption in man, the results of our investigation agree well with those obtained by Pappenheimer and his co-workers on goats, showing an active mechanism of transport in and around the fourth ventricle. It is conceivable that this mechanism of transport may be related to the genesis of hydrocephalus in some cases and further studies of these mechanisms may solve some of the etiological questions of infantile hydrocephalus.

When dealing with cases of expansive hydrocephalus, it is of prognostic and

therapeutic value to be able to determine whether or not the hydrocephalus is communicating. Shunt-operated children with stenotic aqueducts will probably be dependent on a functioning shunt all their lives, while children with communicating hydrocephalus are sometimes able to do well without their shunt after some years (unpublished). Neuroradiological examinations sometimes give uncertain and erroneous results, since the passage of air through the aqueduct is dependent upon many factors, for instance, the inclination of the child's head.

In order to differentiate between communicating and non-communicating hydrocephalus, the dye tests, i.e. the phenolsulphonphthalein urine-excretion tests introduced by Dandy and Blackfan in 1914 [6] and modified by Laurence [9, 10], have proved to be of great value. Probably they too should be interpreted against the background of the experiments by Pappenheimer *et al.* [13]. However these dye tests often demand such high concentrations of the injected substance that the transport maximum is exceeded and other routes of transportation may become significant. The ventricular dye tests may furthermore evoke severe toxic reactions and are considered to be only of limited value [10].

The disadvantages inherent in the dye tests are not inherent in the method presented in this paper. Furthermore the study has clearly shown that the transport of Diodrast from the C.S.F. definitely differs as between cases with communicating and non-communicating hydrocephalus. We believe that the method can be used for the development of a practical and harmless test for pre-operative use in the differential diagnosis between the two main types of hydrocephalus.

In shunt-operated hydrocephalic children in whom there is doubt as to whether or not the shunt is functioning the reported method may be useful as a test to evaluate the patency of the shunt. This test needs only one single needle prick and a fairly small radioactive dose and is easier to perform than other radioisotopic methods for evaluating the patency of the ventriculo-atrial shunt [1, 2, 12]. The method can presumably be further simplified by external counting over the child's head during the hours after injection. Such studies are under way.

In the present study urine was collected through a *permanent catheter*. In infants and young children urinary excretion may be completely suppressed for one to two hours, or even longer following catheterization or other painful stimuli, such as the simple needle prick of the injection [16]. In our cases the diuresis was rather small during the first two hours. However the results indicate that the accuracy of urine collection by catheter is unnecessary and that the urine samples may be collected in tightly fitting plastic bags, which are emptied every half-hour during the first four hours after injection, the exact time of collection being noted.

The active surgical approach to infantile hydrocephalus during recent years requires additional methods of adequate and rapid differentiation between various subgroups. It is imperative that these diagnostic aids should be safe, easy and quick to perform and harmless to the child. In our opinion the method presented fulfils all these requirements. It needs little staff and can be carried out by a specially

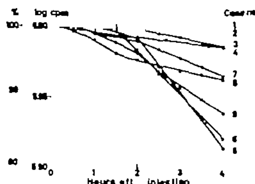


Fig. 1

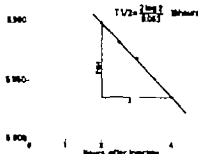


Fig. 2

Fig. 1. Logarithm of retained radioactivity normalized to 10^4 cpm injected, as a function of time. Left-hand axis gives the same radioactivity as a percentage. Retained radioactivity is calculated as the injected radioactivity minus the radioactivity excreted in the urine.

Fig. 2. Calculation of the biological half-time of I^{131} after intraventricular injection of Diodrast into a child with communicating hydrocephalus.

for the cases with stenosis of the aqueduct (cases no 1–4) was considerably delayed. The biological half time for I^{131}-Diodrast was thus 10–24 hours in the cases no 6 and 7 with communicating hydrocephalus compared with 2–12 days in the cases no 1–4 with stenosis of the aqueduct. In the two cases in which the neuroradiological investigation showed no passage of air through the Sylvian aqueduct (cases no 8 and 9) but in which the case histories and the clinical pictures suggested communicating hydrocephalus, rapid excretion of I^{131} Diodrast was obtained with half times of 8–14 hours, as in the cases with communicating hydrocephalus. One child (case no 9) died after the operation, apparently from sepsis, at the postmortem examination no malformation of the aqueduct was found, nor were there any Arnold–Chiari malformations.

Case no 5 is a borderline case clinically radiologically and in relation to the biological half time of the I^{131} and is explained by a narrow and malformed aqueduct.

In the two cases (nos. 1 and 4) in which calculations were performed using the total amount of I^{131} excreted during 48 hours, we obtained identical half-times as when using the first 4 hours after injection.

Discussion

As regards the mechanism of C.S.F absorption in man, the results of our investigation agree well with those obtained by Pappenheimer and his co-workers on goats, showing an active mechanism of transport in and around the fourth ventricle It is conceivable that this mechanism of transport may be related to the genesis of hydrocephalus in some cases and further studies of these mechanisms may solve some of the etiological questions of infantile hydrocephalus.

When dealing with cases of expansive hydrocephalus, it is of prognostic and

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Acid Base and Electrolyte Changes during Exchange Transfusion

by D BODA, GY TÓTH, L. MURÁNYI and E. ECK

Recognition of the significance of incompatibility between maternal and foetal blood types and of hyperbilirubinaemia has led to extensive use of exchange transfusion in paediatrics.

In practice the technique has proved to be almost free from hazards [19]· the babies tolerate it excellently

For our present studies we started with the assumption that exchange transfusion necessarily profoundly influences vital processes and fluid metabolism of the body Even if fresh blood is used, the preserved blood undergoes substantial changes in its composition. In consideration of the differences expected, we thought it was necessary to pproach the problem from several angles. In this report we wish to present our results for the most important factors of systemic homeostasis the pH and bicarbonate value of plasma, its Na^+ and K^+ levels and its calculated osmolarity i.e. factors possibly changing during exchange transfusion.

M terials and Methods

Forty newborn infants requiring exchange transfusion ha e been included in this investigation. Exchange transfusion was carried out 12 times on the 2nd day of life, three times on the 3rd, and 25 times on the 4th, 5th and 6th days of life because of Rh incompatibility in 4 cases, ABO incompatibility in 11 cases, and hyperbilirubinaemia in 25 cases without a known blood type incompatibility

Four types of pretreatment were employed. Eleven patients have not been pretreated at all. Eleven were given 50 ml/kg of ½ isotonic Ringer infusion with 5 per cent glucose. Five patients were gi en 100 mEq/l NaHCO solution and the remaining 13 patients received 165 mEq/l NaHCO in a dose of 50 ml/kg as an intravenous drip infusion administered within 2 to 4 hours. The exchange transfusion was always carried out through the umbilical vein, and the blood samples were taken from there. Blood samples were taken before infusion, before exchange transfusion, t the conclusion of exchange transfusion and 2 hours and 12 hours after exchange transfusion. The blood removed in the course of exchange transfusion was collected, heparinized, and examined for total bilirubin and haematocrit. The blood samples to be used in the acid-base examinations were stored in the presence of NaF and oxalate, air tight, in ice of 0°C until tested not later than 16 hours after sampling. The Na and K determinations were made on plasma containing heparin as an anticoagulant

The pH of blood was determined at 37°C, using biological pH-meter of the Radelkis type and glass electrode, the standard bicarbonate was estimated according to Astrup [1], the plasma N and K were determined by means of a Zeiss flame photometer Serum bilirubin was estimated by the method of Jendrassik & Cleghorn [8].

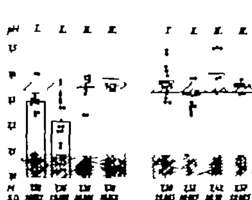


Fig. 1

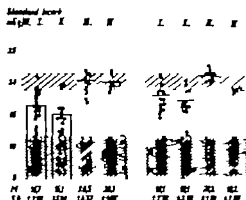


Fig. 2

Fig. 1 (a) Blood pH values of infants during exchange transfusion, without pretreatment (○) and after pretreatment with 50 ml/kg 1/3 Ringer-dextrose (●). (b) Blood pH values of infants pretreated with 100 mEq/l (○) and 165 mEq/l (●) $NaHCO_3$. I: blood pH before exchange transfusion; II: at the end of exchange transfusion, III: 2 hours after exchange transfusion; IV 12 hours after exchange transfusion. M: mean; S.D standard deviation.

Fig. 2. The standard bicarbonate values of the infants shown in Fig. 1 Diagrammatic representation and signs as in Fig. 1.

Results

Acid-base balance before and after exchange transfusion

The numerical data including for the preserved blood are shown in Table I

Blood pH and plasma standard bicarbonate values of the newborn infants receiving exchange transfusion showed that in the majority of cases there was a metabolic acidosis.

The data for the preserved blood shown in the same table make it clear that most of them were extremely acid to a degree practically never encountered *in vivo*. Thus the acidotic infants were given blood of such extreme acidity This is understandable in view of the composition of the stabilizer used (citric acid 0 66 g sodium citrate 2 4 g, dextrose 2.2 g distilled water ad 100 0 pH 5.4). As indicated by the pH data (Fig 1*a*) and the standard bicarbonate values (Fig 2*a*), by the end of the exchange transfusion the initial acidosis significantly increased in severity as a result of the intervention ($t = 6.310$ $P < 0.01$) and in some cases the dangerous zone under pH 7 1 was reached. The same applies to the standard bicarbonate ($t = 2.798$, $P < 0.01$) As the data in column III of Fig. 1 and Fig 2 reveal, the conditions fortunately improve greatly 2 hours after blood exchange, and sometimes even data indicative of a slight alkalosis may be found although in some cases the compensation of acidosis is delayed. Twelve hours later acidosis has been abolished and the acid base balance *improved or normalised, compared* not only with what we found at the end of blood exchange but also with the initial values.

Prevention of the acid-base imbalance in exchange transfusion

The recognition of the serious pH changes noted in connection with blood exchange transfusions suggested that they

TABLE 1 *Blood pH and plasma standard bicarbonate values of newborn infants receiving exchange transfusion and of the blood used for transfusion.*

	n	blood pH		Standard bicarbonate mEq/l
Newborns before blood exchange	22	7.306 ± 0.092	1	16.7 ± 2.62
Transfused blood	22	6.61 ± 0.16	15	6.33 ± 0.48

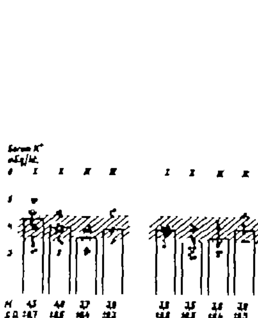
might be prevented by sodium bicarbonate infusion. As the data in Fig 1 and Fig 2 reveal, the pre-blood-exchange acidosis has improved in response to sodium bicarbonate infusion. From the point of view of pH the effect was optimal ($t = 3.102$, $P < 0.01$). Although the mean standard bicarbonate value does not reach the normal limit, the response—especially to larger sodium bicarbonate doses—is still favourable ($t = 1.570$ $P < 0.20$). By the end of exchange transfusion we have found also in this group acidotic values due to the high acidity of preserved blood, but they are much less pronounced than in the cases not pre-treated with sodium bicarbonate ($t = 3.340$ $P < 0.01$). Except for one infant alkalotic even before the exchange transfusion, alkalosis did not develop after the exchange transfusion in spite of the pretreatment with sodium bicarbonate. Thus, it may be stated that by giving sodium bicarbonate infusion the acidosis of the patients receiving exchange transfusion may be corrected. This measure also prevents the acidosis which otherwise occurs as a rule by the end of exchange transfusion without causing the acid-base balance to shift over into alkalosis later

The plasma Na^+ and K levels before and after exchange transfusion

The results for the patients and the transfused blood preparations are presented in Table … It is remarkable that in many cases the infants showed a slight hypernatraemia before exchange transfusion, which we attribute to the circumstance that the tests were carried out at the time when the physiological loss of weight was the greatest. The high Na^+ concentration of the plasma of the transfused blood increased it further causing a marked but transient hypernatraemia. The Na^+ levels changed similarly in the groups not pretreated and in those pretreated with sodium bicarbonate i.e. the administration of the bicarbonate infusion with its relatively high Na concentration did not make the situation worse.

TABLE * *Plasma Na^+ and K levels of newborns before and after exchange transfusion and in the plasma of the transfused blood.*

	n	Plasma Na mEq/l		Plasma K^+ mEq/l
Before blood exchange	22	145.0 ± 7.4	21	4.3 ± 0.7
At the end of blood exchange	19	152.2 ± 6.9	19	4.0 ± 0.6
2 hours after exchange transfusion	13	146.6 ± 6.2	13	3.7 ± 0.2
12 hours after exchange transfusion	10	148.2 ± 10.1	10	4.0 ± 0.3
Transfused blood	22	172.0 ± 9.8	20	6.2 ± 2.2


Fig. 3

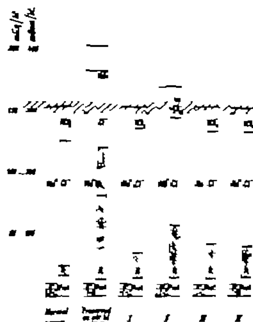

Fig. 4

Fig. 3. Plasma K values of the infants shown in Fig. 1 Diagrammatic representation and signs as in Fig. 1

Fig. 4. Plasma ionogram and osmolarity in the course of blood exchange transfusion. I to IV as in Fig. 1. R unidentified acid residue ion.

TABLE 3 *Percentage changes of the serum bilirubin level.*

	n	Without pretreatment	n	Pretreatment with NaHCO infusion
At the end of exchange transfusion	14	40.5 ± 6.5	13	45.2 ± 10.2
2 hours after exchange transfusion	12	75.6 ± 5.1	12	66 ± 12.6
12 hours after exchange transfusion*	15	77 7 ± 22.4	14	73 ± 21 1
24 hours after exchange transfusion[b]	9	71.6 ± 11.5	8	70.5 ± 9.3
In collected exchanged blood	17	51.7 ± 21.4	15	51.2 ± 19 7

Without the cases of incompatibility
[b] Data exclusively for infants more than 3 days old.

Our normal plasma K^+ levels are 3.5 to 4.5 mEq/l. In this context, the mean plasma K^+ values were normal in both groups before and after exchange transfusion (Fig 3) It is reassuring that no dangerous hyperpotassaemia occurred at the end of the exchange transfusions. In view of the known correlation between acid base balance and plasma K^+ level [4], the infants pretreated with sodium bicarbonate infusion had a lower plasma K^+ concentration than those not getting such pretreatment ($t = 0.7760$ $P < 0.20$) at the end of exchange transfusion ($t = 2.295$ $P < 0.01$) we found hypopotassaemia lower than 3 0 mEq/l in two infants. But this, too, was temporary and in any event it was not severe and did not produce any clinical signs.

Changes in the osmotic pressure of plasma after exchange transfusion

From the Na^+ concentration, as well as from the values of blood sugar and non-protein nitrogen (NPN) we can calculate and examine with reasonable accuracy the changes of the osmotic pressure of blood, and from the values of the protein and Cl^- concentrations we can plot a simplified ionogram. The ionogram columns for the several periods are shown in Fig 4. The columns contain the physiological Ca^{++} and Mg^{++} values, the other data represent the averages obtained in our cases not treated with bicarbonate

It is evident by the end of the exchange transfusion that the stabilizer causes a transient substantial increase in osmotic pressure due to hypernatraemia and hyperglycaemia. The ionograms reveal that in spite of the acidosis there is hypochloraemia by the end of the exchange transfusion which, on the basis of the non-determined anion residue is obviously an organic acidosis, mainly citrate.

Discussion

It is shown clearly by the data outlined above that exchange transfusion is not an indifferent procedure. Several complications including deaths, serious ECG changes and syncope have been described [16, 18] with citrate intoxication, disturbance of the electrolyte balance hypocalcaemia, hyperpotassaemia and acidosis suggested as the cause [3 7 10, 17]. According to our investigations under the conditions employed by us hyperpotassaemia may be ruled out but hypernatraemia with an increase in osmotic pressure will increase nervous disturbances by known mechanisms. We must emphasize in particular the significance of acidosis. The pathological importance of acidosis was recognized long ago. However we have realized the real significance of the problem only since we can make routine tests of the acid-base balance, a procedure which was extremely complicated [10]. In the present circumstances, particular importance is to be attributed to the fact that at low pH there is diminished bilirubin binding by proteins and an increased danger of all damage caused by bilirubin. Povey [14] has recently reported changes similar to those found by us which he obtained by simply following the course of exchange transfusions by pH measurements. He has suggested that the extremely low values themselves may give an explanation of the more serious complications.

As a comment to Povey's communications [14], it has been suggested that concentrated bicarbonate should be administered intravenously at intervals, against acidosis [2]. The same method has recently been advocated by MacRae & Palavradji [9]. On the other hand, Calladine & Gairdner [5] ciriticized this view strongly because of the danger of alkalosis following exchange transfusion. It seems that acidosis may be successfully prevented by our method, i.e., by giving sodium bicarbonate infusion before the exchange transfusion. The administration of the 1/6 mol solution in a dose of 50 ml/kg normalizes the acid-base balance and prevents the acidotic action of exchange transfusion. During the transfusion the excess bicarbonate is partly washed out and the danger of alkalosis is eliminated.

At present, in the treatment of hyper

bilirubinaemia of newborn infants, the efforts to improve the efficiency of exchange transfusions are in the centre of interest [15]. In this respect it has been demonstrated that the infusion of albumin, as suggested by Odell [12, 13] has such an effect. Pretreatment with bicarbonate could be expected to improve the bilirubin-conjugating power of proteins and may be clinically beneficial. We tried to appraise this effect by comparing the bilirubin levels at the end of the exchange transfusion and 2 hours and 12 hours later as well as in the collected exchange blood and the initial blood level. The results are presented in Table 3. In the bicarbonate-pretreated group the bilirubin mean level was higher by the end of the exchange transfusion ($t = 5.055$, $P < 0.01$) while two hours later it was lower ($t = 2.329$, $P < 0.05$). In the other groups the bilirubin level decreased in the same way compared with the pretransfusion value. This may be interpreted as indicating that the alkali infusion mobilizes more bilirubin from the extravasal space, and the subsequent decrease is due to a depletion of the same depots. However the patient material was heterogeneous from this point of view and no definitive conclusions can be drawn.

It may also be asked whether we ought not to use some other stabilizer instead of the acid-citrate-dextrose in the blood preparations. According to Day [6] heparin, in spite of its apparent advantages, is the least suitable for this purpose because it mobilizes fatty acids. The suggestion of using a stabilizer of neutral reaction, containing less citric acid, seems to be the best. These problems as well as the effect of alkalization on the bilirubin binding capacity of serum proteins must first be resolved before it is possible to recommend sodium bicarbonate infusion as routine treatment both for pretreatment of exchange transfusion and for adjuvant treatment of infantile jaundice.

Summary

In connection with blood exchange transfusions carried out on forty newborn infants it has been found that the majority of the newborns requiring exchange transfusion have metabolic acidosis increasing to possibly critical severity by the end of the exchange transfusion. The acidosis, however, is soon normalized spontaneously. Pretreatment with sodium bicarbonate infusion normalizes the pretransfusion shift in acid base balance and prevents the development of a severe acidosis following the exchange transfusion without risking a subsequent alkalosis.

Before exchange transfusion a slight hypernatraemia has often been observed and this increases transiently after blood exchange. Pretreatment with infusion did not modify this phenomenon.

In our material no hyperpotassaemia has been encountered by the end of exchange transfusion. In response to pretreatment with sodium bicarbonate infusion the plasma K^+ level decreased slightly.

By promoting the conjugation of bilirubin to proteins, pretreatment with sodium bicarbonate may presumably improve also the efficiency of the exchange transfusion, this, however requires further proof.

References

1. Astrup P A simple electrometric technique for the determination of carbon dioxide tension in blood etc. *Scand J Clin Lab Invest, 8* 33, 1956.
2. Barrie, H.: pH changes during exchange transfusion. *Lancet, II* 476, 1964.
3. Bolande, R. P Traisman H. S. and Philipsborn H. F Electrolyte considerations in exchange transfusion for erythroblastosis foetalis. *J Pediat 49* 401, 1956.
4. Burnell, J M., Villamil, M. F Uyeno, B. T and Scribner, B. H.: The effect of humans of extracellular pH-change on the relationship between serum potassium concentration and intracellular potassium. *J Clin Invest, 35* 935, 1956.
5. Calladine, M. and Gairdner, D pH changes during exchange transfusion. *Lancet, II* 477 1964.
6. Day R. L.: Indication for exchange transfusion based upon the role of albumin in haemolytic disease of the newborn. *J Pediat, 61*. 832, 1962.
7 Farquhar, J W and Smith, H.: Clinical and biochemical changes during exchange transfusion. *Arch Dis Childh, 33*. 142, 1958.
8. Jendrassik, L. and Cleghorn R. A.: Photometrische Bilirubinbestimmung. *Biochem Z, 289* 1, 1937
9. MacRae, D. J and Palavradji, D Acid-base balance in exchange transfusion. *J Obstet Gynaec Brit Cwlth, 72*. 354, 1965.
10. Miller, G McCoord, A. B., Joos, H. A. and Clausen, S. W *Studies of serum electrolyte changes during exchange transfusion. Pediatrics, 13*. 412, 1954.
11. Lüders, D Experimentelle Untersuchungen über die Bilirubinlöslichkeit. II. Dialyse und Ultrafiltration von Bilirubin. *Z Kinderh, 91* 338, 1964.
12. Odell, G B.: Studies in kernicterus. I. The protein binding of bilirubin. *J Clin Invest, 38* 823, 1959
13. Odell, G. P Cohen, S. M. and Gordes, E. H.: *Administration of albumin in the* management of hyperbilirubinaemia by exchange transfusions. *Pediatrics, 30* 613, 1962.
14. Povey M. J C.: pH changes during exchange transfusion. *Lancet, II* 339, 1964.
15. Rosta, J and Lenkei, P Efficiency of blood exchange in newborn infants. *Acta Paediat Acad Sci Hung 4* 359, 1963.
16. Sacrez, R., Willard, D. and Beauvais, P La surveillance électrocardiographique des exsanguino-transfusions, pratiquées chez les nouveau nés atteints d'incompatibilité foeto-maternelle. *Pediatrie, 16* 657 1961.
17 Taylor, W C., Grisdale, L. C. and Stewart A. G.: Unexpected death from exchange transfusion. *J Pediat, 52*. 69, 1958.
18. Vyhnálek, J and Kaplintál, Z.: Electrocardiographic studies in newborn infants during exchange transfusions. *Ann Paed, 202*. 161 1964.
19. Waltner, K.: Exchange transfusion (Hung.). *Orv. Hetil, 98*. 665, 1957

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REVIEW ARTICLE

Spontaneous Pneumothorax in the Newborn

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Spontaneous pneumothorax is said to be more common in the newborn period than at any other time during childhood. Although an infrequent cause of respiratory distress it is important to recognise and assess this condition as in cases of tension pneumothorax prompt treatment can be life saving. Chernick & Avery [1] reviewed the subject in exemplary fashion and added 15 case histories to the literature.

The detection of pneumothorax in newborn infants will depend on a high index of suspicion plus a knowledge of the predisposing factors and physical findings. Confirmation of the diagnosis rests on the availability of X ray facilities or direct aspiration in cases of emergency.

The object of this communication is to record our experience with spontaneous pneumothorax (unrelated to hyaline membrane disease) in newborn infants and to draw attention to an important diagnostic feature.

Pneumomediastinum is included under our title of pneumothorax as it is probably a variant of the same underlying pathology [8].

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Clinical Material

Seven cases of symptomatic spontaneous pneumothorax were seen over a period of one year. Six of these infants were born in the Teaching Hospitals of the University of Cape Town and the seventh infant was referred to the neonatal respiratory unit from a country hospital. The incidence of symptomatic spontaneous pneumothorax of the newborn in the University of Cape Town Teaching Hospitals was 0.06 % (10 000 live births). During the same period 10 cases of pneumothorax complicating hyaline membrane disease were diagnosed. The incidence of pneumothorax in hyaline membrane disease was 16.4 %, which is comparable to our previous experience [7]. The hyaline membrane cases will not be discussed in this communication.

The clinical details of the 7 infants are set out in Table 1. In none of them were resuscitative measures employed at birth and they all survived.

The radiological findings are shown in Table I. Case 7 had the accepted radiological picture of meconium aspiration [10]. Some degree of pneumomediastinum was present in all except Case 2. Consolidation of one lobe was seen in Case 6.

Tension pneumothorax developed in Case 2 and required emergency needle-aspiration. This was followed by under water drainage through a chest catheter for 12 days, with complete recovery. Antibiotics were used when infection was suspected or thought to be an additional risk.

TABLE 1 *Details of infants with spontaneous pneumothorax and pneumomediastinum.*

Number and initials	Sex	Race	Birth weight kg	Apgar rating	Obstetrical factors	Delivery	Signs first noted	Radiological findings: Thorax	Radiological findings: Mediastinum	Treatment	Cleared in
1 B. T	M	Col.	2.39	3/10	Ruptured membranes 48 hours	Vacuum	½ hr	R & L	+	Antibiotic	4 days
2. B C.	F	White	3.51	7/10	Postmature. Foetal distress. Meconium liquor + +	Forceps	½ hr	R (tension)	—	T be drain R Antibiotic	13 days
3. B. J	M	White	2.90	7/10	Rapid delivery of body	Normal vertex	1 hr	L	+	None	2 days
4 B. A.	M	Col.	2.83	—	Postmature. 15 min delay with head	Breech	1 hr	L	+	None	6 days
5. B. R	M	White	2.87	5/10	Face presentation	Forceps	1 hr	L	+	None	3 days
6. B. B.	M	Col.	2.88	7/10	Normal	Normal vertex	½ hr	—	+	Antibiotic	4 days
7 B. W	M	White	4.17	—	Cord around neck. Facial petechiae. Meconium liquor + +	Normal vertex	Birth	L	+	Antibiotic N HCO$_3$	14 days

Consol. L.U L.

Clinical Presentation

The clinical signs and their relative frequencies are summarised in Table 2. Tachypnoea, up to 130/min, was seen in all the infants. A prominent chest bulge was present in 6 cases. The bulge was on the same side as a unilateral pneumothorax in Cases 2, 3 4 and 5 In Cases 1 and 7 there was central sternal bulging. In Case 6 no bulge could be detected. The presence of a chest bulge in association with other signs led to the correct diagnosis prior to radiology in five instances.

Central cyanosis was present shortly after birth in 6 cases. The cyanosis was prolonged in Cases 2 and 7 in the others it was of a few hours duration. Recession was a constant finding but of a much lesser severity than that seen in infants with hyaline membrane disease.

Five infants had persistent grunting and decreased air entry Diminished air entry on the side of the chest bulge was found in 4 cases. In the fifth, the air entry was initially decreased on the side opposite to the pneumothorax but became equal after 2 hours. Unusual irritability and restlessness was present in 4 cases. Cardiac signs were not helpful in this series, a shift of the cardiac impulse being detected

TABLE — *Clinical presentation.*

RR > 60	100 %
Chest bulge	
Cyanosis	86 %
Recession	
Grunting	71 %
Decreased A	
Irritability	57 %
Cardiac signs	43 %

TABLE 3 *Acid-base biochemistry*

Number and initials	Age in hours	pH	PCO mm Hg	Base excess mEq/l	Buffer base mEq/l	Standard HCO_3 mEq/l	Actual HCO_3^- mEq/l
1 B. T	12	7 457	25.0	−2.6	47 0	22.0	17.5
	15	7 460	26.0	−4.4	44.8	20.5	17 0
2. B. C.	2	7 184	53.0	−9.3	38.5	17.2	19.2
	4	7 400	38.0	−1 0	48.0	22.2	22.0
4. B. A.	12	7.262	30.8	−7.2	36.8	18.2	17.0
	19	7.372	39.5	−2.1	45.5	22.2	22.1
5. B. R.	2½	7 370	34.0	−5.0	40.6	20.0	19.0
	13	7.376	38.9	−2.2	47 0	22.2	22.0
6. B. B.	8	7.395	35.5	−2.8	42.6	21 6	21.0
7 B. W	31	7 150	48.6	−11.2	33.4	18.3	16.4
	47	7.347	46.2	−0.6	44.9	23.3	24.4

only in one child. In two others the heart sounds were faint, with occasional crepitations over the sternum.

Biochemistry

The acid base findings in 6 of the infants are given in Table 3

Case 1 presented with a respiratory basosis associated with a very high respiratory rate. The pCO_2 rose to 35 mm Hg at 77 hours. In Case 2 there was prompt return from a combined acidosis to normal values after needle aspiration of a tension pneumothorax. No values were obtained in Case 3 Cases 4 5 and 6 were essentially normal biochemically Case 7 was a severely ill infant with meconium aspiration who was given sodium bicarbonate intravenously to correct the non respiratory acidosis. The arterial oxygen tension was measured in this infant only and was found to be 72 mm Hg

Discussion

The incidence of symptomatic spontaneous pneumothorax of the newborn in this series is 0 06 %. This figure corresponds to those of Harris & Steinberg [4] Howie & Weed [6] Lubchenco [8] and Chernick & Avery [1] It has been pointed out that the radiological incidence is much higher than would be suspected from clinical signs. Radiological facilities are not available in all our maternity homes with the result that only moderately and severely ill infants were referred for investigation. Emery [2] has estimated that a variable amount of interstitial air causing no symptoms, would perhaps occur in 1 out of every 200 normal full term infants, i.e. 0.5 %.

None of the infants in the present series was premature by weight (average birth weight 3.28 kg) We have no experience of pneumothorax in premature infants other than as a complication of hyaline membrane disease

There were many similarities between the present series and those reported by Howie & Weed [6] and Chernick & Avery [1]. The vast majority of their infants had birth weights over 2.5 kg in contrast with the hyaline membrane group There was a strong male preponderance. In most instances the onset of distress occurred shortly after birth, if not in the delivery

room itself. Several infants were born by complicated deliveries. In these cases there is presumably a greater risk of aspiration of foreign material.

The association of meconium and mucus aspiration in the pathogenesis of pneumothorax has been discussed by Chernick & Avery [1] and Emery [2]. The development of pneumothorax after over vigorous resuscitation with positive-pressure respiration clearly belongs to a separate etiological group [., 9].

In the present series the presence of a chest bulge, either unilateral or central, has been a prominent feature. The finding of a unilateral chest bulge, decreased air entry on the same side, tachypnoea and cyanosis is virtually diagnostic of a pneumothorax. Heald & Wilder [5] recorded a marked precordial bulge with almost absent heart sounds in infants with pneumomediastinum. Prosser [11] mentioned over-distension and fixation of the chest as signs of interstitial emphysema. Vines [12] also recorded decreased air entry and distant heart sounds,but did not mention bulging of the chest wall. Radiological confirmation of a clinical diagnosis of pneumothorax is imperative.

Several infants of the present series displayed unusual irritability and more attention should perhaps be paid to this sign. It seems to be an important feature of pneumothorax in premature infants [8]. In this series the cases with irritability (Cases 1, 4, 5 and 7) had the highest respiratory rates, 60–120/min. It is uncertain whether this should be attributed to anoxia or to the irritant effect of air within the mediastinum. Extra-pulmonary air within the chest is probably painful or at least a source of discomfort. Lubchenco noted an improvement in the irritability after oxygen administration [8]. This may have been an expression of the more rapid absorption of loculated air while breathing higher concentrations of oxygen [1]. Case 7, while breathing 50 % oxygen, showed marked irritability and tachypnoea and had a pO_2 of only 72 mm Hg.

A high incidence of pneumomediastinum is reported in this series. This probably represents individual variation in assessment of small amounts of mediastinal air. A high incidence of pneumomediastinum is not surprising as it has been shown experimentally that the mediastinal pleura requires less tension to rupture than the visceral pleura [3]. The various sites of collection of air were illustrated diagrammatically by Kottler *et al.* [7].

Except for those with tension pneumothorax and meconium aspiration the infants in this series showed little disturbance of their acid-base balance. The only other available figures for pH, pCO_2 and base excess are those of 2 cases recorded by Prosser [11]. One infant had hyaline membrane disease and the other a tension pneumothorax with acid base figures similar to those of Case 2 in this series.

The principles and practical aspects of treatment have been fully discussed by Chernick & Avery [1]. Vines [12] drew attention to the importance of negative suction drainage where there is underlying lung disease. This was not applied in our patient with tension pneumothorax but it would probably have shortened the period of absorption.

Summary

The obstetrical, clinical, radiological and biochemical findings in 7 cases of spon-

taneous pneumothorax of the newborn are presented. All the infants survived. Attention is drawn to the presence of a chest bulge as a prominent physical sign in the diagnosis of pneumothorax. The significance of irritability in association with a rapid respiratory rate is discussed.

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References

1. CHERNICK, V. and AVERY, M. E.: Spontaneous alveolar rupture at birth. *Pediatrics* 32: 816, 1963.
2. EMERY, J. L.: Interstitial emphysema, pneumothorax and "air block" in the newborn. *Lancet*, 1: 405, 1956.
3. HAMMAN, L.: A note on the mechanism of spontaneous pneumothorax. *Ann Int Med*, 13: 923, 1939.
4. HARRIS, L. E. and STEINBERG, A. G.: Abnormalities observed in the first 6 days of life in 8716 live-born infants. *Pediatrics*, 14: 314, 1954.
5. HEALD, F. and WILDER, T. S.: Pneumomediastinum in the newborn infant. *J Pediat*, 34: 225, 1949.
6. HOWIE, V. M. and WEED, A. S.: Spontaneous pneumothorax in the first ten days of life. *J Pediat*, 50: 6, 1957.
7. KOTTLER, R. E., MALAN, A. F. and HEESE, H. de V.: Respiratory distress syndrome in the newborn. Radiological aspects. *S Afr J Radiol*, 2: 35, 1964.
8. LUBCHENCO, L. O.: Recognition of spontaneous pneumothorax in premature infants. *Pediatrics*, 24: 996, 1959.
9. OTTALAY, A. G. and BEARD, A. G.: Tension pneumothorax: possible etiological rôle of high intratracheal pressure. *J Pediat*, 63: 530, 1963.
10. PETERSON, H. G. and PENDLETON, M. E.: Contrasting roentgenographic patterns of the hyaline membrane and fetal aspiration syndromes. *Amer J Roentgenol*, 74: 800, 1955.
11. PROSSER, R.: Interstitial emphysema in the newborn. *Arch Dis Child*, 39: 236, 1964.
12. VIKES, R.: Suction drainage for pneumothorax in neonates. *Med J Aust*, 1: 201, 1963.

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CASE REPORT

Upper Limb Fractures as a Complication of Vaccination

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The purpose of this paper is to record two patients in whom fracture of the upper limb followed routine vaccination.

Case Histories

Case 1

A. S. was a five-month-old boy who was first seen in the Casualty Department of this hospital, when his parents had become worried about his right arm. This had become swollen following a vaccination three days previously The history given by the parents, was that after his first injection of "triple vaccine" his arm had become swollen and he had cried when attempts were made to move the arm. The following morning a click was felt by the mother when she moved the arm and both parents became so anxious that they took the child back to see their own doctor They were reassured and told that this was just the normal reaction to vaccination and that it would soon get better The following day the arm was found to be slightly more swollen and still very tense and the child continued to fret when the arm was moved. As a consequence of these symptoms, the child was brought to the hospital.

This baby was nine weeks premature at birth and weighed 2 lb. 4 oz. On admission he weighed 10 lb. 5 oz. and he had been perfectly healthy apart from one attack of tonsillitis which had occurred about two months previously He was the youngest of four boys, all of whom are alive and well. The father was a representative for children s care society and both parents seemed to be very concerned about the condition of their child. They denied that he had ever been exposed to any form of trauma.

On examination the child appeared to be well and he was apyrexial. He did not seem to be suffering any discomfort while being held by his mother He kept his right arm still, but was able to move his fingers. The arm felt warm and tense and was swollen from the shoulder to the wrist. There was no evidence of any nervous or vascular injury to be found. All attempts at passive movement of the right arm were resisted x Ray examination of this arm revealed a fracture of the shaft of the right humerus and a transverse fracture of the distal end of the right radius.

In view of the multiplicity of his injuries he was admitted to hospital for further observation. His arm was immobilised in a "U" slab plaster of Paris splint. A radiological survey of his skeleton performed the following day did not reveal any evidence of epiphyseal or metaphyseal damage other than that in his right arm, and it was felt that this did not represent a case of the "Battered Baby" syndrome [4 5]. He was discharged home four days after admission. The plaster slab was removed from the arm four weeks later at which time an x-ray examination showed that there was abundant callus around the fracture sites. On clinical examination the fractures were united. He was discharged from the orthopaedic de-



Fig. 1. Case 1 Radiograph on admission t hospital. Note fracture of shaft of right humerus and fracture of lower end of right radius

partment, but was later admitted to a children s hospital when it was discovered that he had primary pulmonary tuberculosis. He improved on I.N.A.H. and ppears to be doing very well at the present date

Case 2

E C., a seven year-old boy was seen in the Casualty Department at this hospital complaining of pain in his left wrist. Four days previously he had been vaccinated at the School Clinic and he stated that t this time the nurse had held his left wrist. The day after the injection the arm became very painful and he came up to hospital. There was no history of a fall and, even on close questioning, it was impossible to obtain a history of any other form of trauma.

On examination there was some swelling and bruising of the lower end of his left radius with slight angulation of the distal fragment. There was no evidence of any other injury and no nervous or vascular damage could be detected. He was apyrexial. An x ray examination revealed the presence of a greenstick fracture of the lower end of his left radius with angulation. A short arm plaster of Paris cast was pplied and this was removed after three weeks. At this time the fracture was united in a satisfactory position and the patient was discharged.

Differential Diagnosis

In both of these cases there is a similar history of routine vaccination followed by swelling tenderness and loss of mobility in the vaccinated arm Virus involvement of bone is rare and only the smallpox virus and the vaccinia virus are known to have affected bone in man [1 2 3]. Case 1 was not exposed to either of these viruses, although the possibility of this is



Fig. 2. Case 2. Fracture of the lower end of the left radius with forward angulation of the distal fragment. Note similarity of this fracture to the radial fracture in Fig. 1

not so certain in Case . The absence of fever and the x-ray appearances characteristic of fracture are against an infective aetiology for these lesions.

One of the features which is said to aid in the diagnosis of the Battered Baby" syndrome [4] is conspicuous absence of significant trauma in the history. This is true of the two cases reported in this paper but the absence of multiple epiphysial injury characteristic x ray changes, elevated temperature and raised white cell counts are, however all against such a diagnosis. The bony changes in the Battered Baby" syndrome are juxta epiphysial and multiple, whereas here in the first child both injuries were dia physial and in the second child the injury was high in the metaphysis.

Congenital variations of the pain threshold may be excluded in these two cases since it was this very symptom which first made these patients come to hospital. If the history of the absence of other trauma is accepted, some relationship must exist between the injuries observed in these two patients and the act of vaccination.

Discussion

The commonest site used for vaccination, for cosmetic reasons, is the posterolateral surface of the arm, overlying the insertion of the deltoid muscle. The methods in use t the present time vary from light scarification to intramuscular injection and it may often become necessary to steady or restrict the arm. There may be considerable disproportion between the strength of the patient and that of the vaccinator under these circumstances since the greatest numbers of immunisations are performed on young children.

The only form of trauma admitted in either of the cases reported in this paper was that of vaccination. Both children appeared to be well systematically and in neither was it possible to discover any history of abnormal bone fragility. It is difficult therefore to explain these injuries and it is suggested that over vigorous attempts to immobilise the arm of a rather apprehensive patient prior to and during vaccination might result in an injury of this nature. It is interesting that the initial swelling was interpreted as a normal sequence of vaccination in one of the cases.

No other reference to the possibility of this complication following vaccination has been found in literature. It is hoped that in the presentation of these two cases, awareness of the possibility of this type of injury may be ppreciated and so prevented in the future.

Summary

Two cases of fracture of the upper limbs are reported in children following vaccination.

A possible mode of production of these injuries is discussed.

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References

1. Cockshott, P and MacGregor, M. *Quart J Med*, 27 639, 1955.
2. Cochran W Connolly J H. and Thompson L D *Brit Med J II* 285, 1962.
3. Elliott, W D: *Lancet, II* 1053, 1958.
4. Griffiths, D Ll. and Moynihan F J *Brit Med J II* 1558, 1963.
5. Gaffey J *Brit J Radiol*, 30 225, 1957

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CASE REPORT

Rutherfurd s Syndrome

A Familial Oculo Dental Disorder

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In 1931 Rutherfurd [11] reported the occurrence of combined corneal dystrophy hypertrophy of the gums and failure of tooth eruption in three generations of the same family In 1962 a boy aged 3 years was brought to our attention with this same triad and on examining his family tree it seemed very probable that its first three generations were identical with those described by Rutherfurd. Unfortunately the case records of 1931 had been destroyed and it is therefore impossible to be certain that these two families are one and the same but the similarities of age sex and disability are such that Dr Rutherfurd and we are satisfied that both reports concern the same family enlarged since 1931 by the addition of three new affected members.

This family s disability seems to be unique and an extension of Rutherfurd original report may therefore be of interest.

Case Histories

The family tree is shown in Fig. 1

V 1 Aged 3 years. Only 4 teeth (the upper left lateral incisor both lower central incisors and the left lower lateral incisor) had

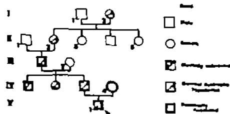


Fig 1 Family tree The arrow indicates the propositus.



Fig 2. Teeth and gums of propositus (V 1). One upper incisor is not shown on the photograph.



Fig. 2. (a) X ray of mandible showing unerupted teeth. (b) X ray of both jaw (left side) show absorption of deciduous teeth which remain unerupted. Permanent dentition present.

erupted (Fig 2) and there were bilateral, diffuse, curtain like opacities occupying the upper half of each cornea. Examination f the skin, hair finger and toe nails, and indeed general physical examination showed no abnormalities. His mental development did not seem overtly abnormal, but he was prone to destructi e behaviour outburst of intense jealousy and similar emotional disturbances.

There was no impairment of his ability to sweat; the sweat sodium concentration was 47 mEq/l, normal for his age [2].

Serum calcium and inorganic phosphat urinary aminoacid chromatography X ray f the limbs and skull revealed no abnormalities. Full mouth X-rays (age 5 yrs.) (Figs. 3a and 3b) confirmed the presence of all his deciduous teeth and all the permanent teeth except the third molars which normally start to develop at nine years of age. Although the deciduous teeth had not erupted they showed evidence of root resorption which was in advance of that expected for his age. There was evidence of dent gerous cyst formation around three of his four first permanent molars. An attempt at chromosomal analysis using venous blood was technically unsatisfactory and permission for a second atter pt was efused

IV 3 The father of V 1 aged 27 years, had been educated at a school for the education ally subnormal but is now regularly em ployed as a painter On examination he had similar corneal opacities to his son s, though denser occupying the upper half of each cornea (Fig 4) His teeth had never erupted from his hypertrophied gums (Fig 5) but two unerupted ones were surgically removed during treatment for a mandibular abscess

N ther abnormalities were found; in particular the ther ectodermal structures were normal. Sweating was normal; the sodium concentration was 70 mEq/l, high normal for his age. A satisfactory prepara tion of venous blood showed a normal male chromosomal pattern.



Fig. 4. Subject IV 2; note the dense curtain-like corneal opacities involving the upper half of each cornea.



Fig. 5. Gums of propositus father (IV 3).

IV 2 Age 29 years. This patient had been in various mental hospitals since the age of 5 years and is currently confined because she is mentally retarded (I Q 30) and has destructive tendencies. Information kindly supplied by Dr D R. K. Street confirmed that she too had corneal pacities, hyper trophied gums and no erupted teeth.

IV 1 Age 32 years. He is also in a mental hospital with mental etardation, epilepsy and is prone to violent outbursts. He has been personally examined and showed neither the corneal nor dental abnormalities noted in other members of the family

An agar plat palm print showed a normal sweat-salt concentration and chromosomal analysis using enous blood showed a normal male karyogram.

This patient corresponds to the 8-month-old infant mentioned in Rutherfurd (1931) report though he was not known t that time to be mentally retarded.

III 1 Age 53 years. Examination revealed that he had even denser corneal opacities than his son (IV 3) and his vision was p-preciably impaired. A number of teeth were unco ered by surgical operations in earlier lif but none are now isible the gums were very hypertrophic. N abnormality of skin, hair or finger and toe nails was found but permission for further examination and in estigation was refused. There was no ap-rent intellectual abnormality

II This patient teeth, eyes and gums were reported, according to her son (III 2) to show bnormalities similar to his own. She was seen and examined by Rutherfurd [11] when she was 46 years old and these abnormalities were noted then.

I 2 This patient was reported in 1931 (by II 2) and currently (by III 2) to have the same ocular and dental abnormalities as other family members. All other members of the family are reported to be normal and this is probably reliable information as the abnormality is such that it is easily bserved and w ll known to the family

Discussion

A number of genetically determined disorders have been described in which failure of eruption of teeth is a feature These can be divided into three groups: (1) the ectodermal dysplasias, ( ) cleidocranial dysostosis and (3) fibromatosis gingivae.

The mechanism of the failure of normal dental development differs in each case In the ectodermal dysplasias hidrotic [9] and anhidrotic [1 6 1] there is failure of development or suppression of the dental lamina w th the result that

few if any teeth are formed. In cleido-cranial dysostosis the teeth develop normally and there is no suppression of the dental lamina there may indeed be multiple supernumerary teeth present. However there is a failure of bone remodelling which delays eruption of the deciduous dentition and may cause retention of the whole of the permanent dentition within the bone. In fibromatosis gingivae [13] the increased thickness of the epidermis of the alveolar mucosa acts as a barrier to eruption of the teeth, which as in cleido-cranial dysostosis, develop normally but do not erupt.

Corneal abnormalities are not usually a feature of any of these syndromes but a few reports linking ectodermal disease with ocular lesions have appeared. An interesting family described by Kline *et al.* [7] exhibited independently inherited anhidrotic ectodermal dysplasia and a form of corneal dystrophy Some members had one and some had both anomalies unlike the present family whose ocular and dental abnormalities always occurred together Moreover few teeth are formed in anhidrotic ectodermal dysplasia and these erupt normally in contrast the teeth of the present family have formed normally but have not erupted.

Friederich & Seitz [3] described a patient with dysplasia of hair and nails and failure of sweat and lacrimal secretion, the latter resulting in recurrent superficial corneal erosion, the patient's teeth were normal. There is no evidence that any of our cases have ever had any corneal ulceration. The steadily increasing corneal opacity of the present cases is more comparable with a patient reported by Greither & Tritsch [4], who had anhidrotic ectodermal dysplasia with cloudiness of the lens and cornea, but also hyperkeratosis of the palms and soles and degeneration of the macula.

The defects in the present family are clearly inherited as an autosomal dominant gene with a high degree of penetrance By contrast, anhidrotic ectodermal dysplasia is usually inherited as a recessive gene, probably sex linked [6] but occasional families may exhibit autosomal recessive or perhaps incompletely penetrant, dominant heredity [1 7] Cleido-cranial dysostosis is inherited as a dominant gene and it is probable from the published cases that hidrotic ectodermal dysplasia [9] and fibromatosis gingivae [10] are similarly transmitted.

Chromosomal abnormalities have not been described in this group of conditions though some cases of Down's syndrome are known to have hypodontia [14] Two members of the present family one with oculo-dental abnormalities and one without (but mentally retarded) showed no abnormality of chromosomal number or structure.

As two members of the present family are severely mentally retarded (IV 1 and IV 2) and in view of the ectodermal origin of the brain, it is tempting to suppose that this may also be part of the same condition but, of these two patients, only one has the oculo-dental anomalies (IV 2) and earlier generations do not show gross evidence of mental subnormality If the neurological disorder is inherited, it may be transmitted independently of the other abnormalities. However as a single inherited anomaly may express itself in different fashions in different individuals it is impossible wholly to exclude the

concept that only one gene may be responsible.

Halperin & Curtis [5] reported one case of anhidrotic ectodermal dysplasia associated with mental retardation, and mental deficiency is also associated, together with dental abnormality in the Bloch-Sulzberger syndrome (Incontinentia Pigmenti [2]) an ectodermal defect characterised by blistering of the skin with pigmentation of the scars. This may be associated with a variety of other defects, including failure of development of many teeth, multiple abnormalities of development of the eyes (but not clouding of the cornea) and paralyses. Some of these associated defects occur in the absence of the skin lesions in members of affected families but the ocular and dental abnormalities are different from those in the family described here.

The diagnosis of fibromatosis gingivae is acceptable from the dental standpoint but histological confirmation (so far not obtained) would be preferable However ocular abnormalities have not been described in this condition and none of the other syndromes seem applicable to the present cases.

Summary

The association of corneal dystrophy hypertrophy of the gingivae and failure of tooth eruption is described in five generations of a family believed to be the same one as that described by Rutherfurd in 1931 The abnormalities are inherited together through an autosomal dominant gene but no gross abnormalities of chromosomal size, shape or number have been identified.

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References

1 BERNARD, R., MARSTRAGGI, P ODDO, G and AUDIBERT, G Étude génétique et clinique d'une dysplasie ectodermique familiale (hérédité dominant hologyne). *Pédiatrie, 14* 848, 1959
2. BUTTERWORTH, T and STREAN L. P Clinical Genodermatology Williams and Wilkins, Baltimore, 1962.
3. FRIEDERICH, H L. and SEITZ, R.: Über eine Form der ektodermalen Dysplasie unter dem Bilde der Pili torti mit Augenbeteiligung und Störung der Schweiss-Sekretion. *Derm Wschr 131* 277 1955.
4. GARTNER, A and TAUSCH, H.: Über einen Fall von anhidrotischer ektodermaler Dysplasie mit nahezu vollständiger Alopecie, transgredienten Palmar Plantar Keratosen, Maculadegeneration sowie anderen Augenstörungen, Zahnanomalien und einem Pseudo-Klinefelter Syndrome. *Arch Klin Exp Derm 216* 50, 1963.
5. HALPERIN, S. L. and CURTIS, G M.: Anhidrotic ectodermal dysplasia associated with mental deficiency *Amer J Ment Defic, 46* 459, 1942.
6 JEPPESEN H. G.: Hereditary ectodermal dysplasia of anhidrotic type report of 3 cases in boys aged three to four months. *Acta Paediat Scand, 51* 712, 1962.
7 KLINE, A. H., SIDBURY J B. and RICHTER, C. P The occurrence of ectodermal dysplasia and corneal dysplasia in one family *J Pediat, 55* 355, 1959
8 MCKENDRICK, T Sweat sodium levels in normal subjects, in fibrocystic patients and their relatives and in chronic bronchitic patients. *Lancet, 1* 183, 1962.
9 ROBINSON G. G MILLER, J R. and BENSON

son J R.: Familial ectodermal dysplasia with sensori-neural deafness and other anomalies. *Pediatrics*, 30 797 1963.
10. Rushton, M. A.: Hereditary or idiopathic hyperplasia of the gums. *Dent Pract*, 7 136, 1957
11. Rutherford M. E. Three generations of inherited dental defect. *Brit Med J II* 9, 1931
12. Stephens, G H.: Anodontia: two cases of different aetiology *Oral Surg 16* 73, 1963.
13. Thoma, K. H., Goldman H. M.: *Oral Pathology* C. V Mosby Co., St. Louis, 1960.
14. Witkop C. J Dental Genetics 1959 *J Amer Dent Ass, 60* 564, 1960.

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CASE REPORT

Ataxia Telangiectasia

by BJARNE SMEBY

The first case of ataxia telangiectasia was reported in 1941 by Louis-Bar [7] During the last 5 years the syndrome of ataxia telangiectasia has become a clearly defined pathological entity [1]. The disease starts as a progressive cerebellar ataxia in infancy and later progressive oculocutaneous telangiectasiae develop. The patients often present with pseudoophtalmoplegia and are liable to acquire sinopulmonary infections. Boder & Sedgwick [1] found a total of 110 cases reported in the literature and have described the disease in detail.

The most common cause of death in ataxia telangiectasia is a combination of bronchiectasis and pneumonia, as a rule at an early adult age. In some cases malignant processes, localized to the lymphoid tissue have been found at autopsy [1 8]

As a rule pneumoencephalography reveals cerebellar atrophy The most characteristic histological finding in the central nervous system is a primary chronic diffuse degeneration of the cerebellar cortex, mainly located to the Purkinje cell layer the granule cell layer and, to less extent to the basket cell layer Changes in the spinal cord have not been demonstrated [1] In 3 out of 5 post mortem examinations venectases in the white substance of the cerebellum and in the leptomeninges have been demonstrated. No vascular changes have been observed in the lungs.

Ataxia-telangiectasia is assumed to be a hereditary metabolic disorder but the metabolic anomaly is obscure. Transmission is said to be autosomal recessive. Fireman *et al.* [4] and Harboe [6] have recently demonstrated a specific dysgammaglobulinemia with a reduced amount of or absence of serum γ-globulin in ataxia-telangiectasia.

A few cases have previously been described in Scandinavia [2, 3 5]. The following five cases have recently been studied and are previously unreported.

Case Histories

Case 1

T I born 1952. This boy was 8 years old when admitted. There had been no similar disease in the family and pregnancy and delivery had been uneventful. Birth weight was 3800 g, length 52 cm. There were no neonatal complications. Psychomotor development during the first year of lif was normal. The patient talked at the normal age and walked at 1 year of age, but from age 15 months his gait became progressively more unsteady The eyes had been red from the age of 2–3 years. The patient had had pneumonia 5 times and frequent attacks of bronchitis.

Findings On admission the length was

123 cm and the weight 23.5 kg Telangiectasiae were present in the bulbar conjunctivae (in the nasal corners of the eyes), in the ears and on the cheeks. Patches of poorly pigmented skin were observed in the face but there were no changes of the hair He presented nystagmoid eye movements and when focusing on near objects, the eyes rotated upwards. He had a fixed facial expression and spoke slowly and indistinctly He had a stooped carriage, narrow shoulders and a protruding abdomen. At 8 years of age the gait was uncertain and slow with the legs widely apart. A 10 years of age the patient had difficulty in walking without support. He presented reduced muscular tonus and choreoathetotic movements of the face and upper extremities, but no spasticity or rigidity The co-ordination was uncertain and Romberg's test positive. Dysdiadochokinesis was present and the movements were slow Sensibility to painful stimuli and touch, joint sense and stereognostic sense were normal.

The deep reflexes, which had previously been normal, were impaired but symmetrically present; the plantar reflexes were normal. Examination by a psychologist at 7 years and 10 months of age: Intelligence in accordance with the age, but the patient has difficulties in making use of his abilities. EEG at 2 years of age and at 10 years of age: No definite pathological findings. Paper chromatography of the urine: Normal amino acid pattern.

Immunologic examination revealed a lack of γ-globulin The following technique was used: "The patients' sera were tested by diffusion in gel analyses using antisera that reacted specifically with γ_G, γ_A and γ_M-globulin respectively Two different anti γ_A antisera were used, with identical results. No abnormalities were detected with regard to the γ_G- and γ_M-globulins.

Case 2

G. B., born 1952. On admission this boy was 11 years old. There had been no similar disease in the family and pregnancy and delivery had been uneventful. Birth weight was 3550 g length 50 cm. There were no neonatal complications. Psychomotor development during the first year of lif was normal. The patient talked at the usual age and walked alone at 13 months of age. At 15 months of age the gait became unsteady and the balance progressively poorer The patient' eyes had been red from age 4–5 years. At 15 months he had bronchitis and otitis lasting one week but he had not been particularly susceptible to colds.

Findings. On admission the length was 132 cm and the weight 32 kg. Telangiectasiae were present in the bulbar conjunctivae, disappearing at the margin of the cornea, and also in both ears and over the left mandible. The skin was poorly pigmented under the eyes, over the root of the nose, in front of the ears and on the ear lobes, and in patches on the neck. There were no changes of the hair The patient presented atactic eye movements and convergent strabismus and when focusing turned his eyes upwards. He had a rather immobile facial expression and spoke slowly and indistinctly He had a stooping carriage and walked unsteadily There were choreoathetotic movements in the face and the upper extremities, but no spasticity or rigidity The co-ordination was uncertain and Romberg test positive. Dysdiadochokinesis was present and the movements were slow Sensibility to painful stimuli and touch, joint sense and stereognostic sense were normal.

The deep reflexes, originally normally developed, were impaired but symmetrical, plantar reflexes were normal. X ray examination of the cranium gave negative findings. EEG (2 examinations): "Moderate general cerebral dysrhythmia, most pronounced over the right temporal region; no epileptogenic activity" Examination by a psychologist at 8 years of age: "The patient reasoning ability appears remarkably good but his central disorder makes it very difficult for him to make use of it. Paper chromatography of the urine: Normal amino acid pattern. Immunologic examination revealed lack of γ_A-globulin.

Case 3

L. J born 1953. This girl was 10 years and 2 months old when admitted. There was no family history of similar disorder. The mother was operated for mammary cancer following pregnancy in 1964. Pregnancy had been uneventful but the child was delivered by forceps. She had the cord round the neck and was given oxygen for a short time. Birth weight was 3270 g, length 52 cm. There were no other neonatal complications. Psychomotor development during the first year of life was normal. The patient talked at the normal age and walked at 16 months of age but easily lost her balance and in spite of regular physiotherapy the balance became steadily poorer. When she was 5–6 years old the parents noticed that she was red under the eyes. A 9 years of age she had bronchitis lasting for 6 weeks. Otherwise, she had only had minor respiratory infections.

Findings. On admission the length was 134.5 cm and the weight 25.9 kg. Telangiectasias were present in the bulbar conjunctivae disappearing at the margin of the corneas, and also around both eyes, especially in the nasal corners, sparsely in the outer ear and in the right cubital region. The skin was poorly pigmented on both temples and moderate facial seborrhoea was present, but no hair changes. The patient presented nystagmoid eye movements and turned her eyes upwards when focusing, but had no strabismus. Fixed facial expression and slow and indistinct speech was also observed. She had marked stoop, with the head and shoulders falling forwards, and walked un steadily. There were choreoathetotic movements of the face and upper extremities, but no spasticity or rigidity. Co-ordination was uncertain and Romberg's test positive. Dysdiadochokinesia was present and the movements were slow. Sensibility to painful stimuli and touch, joint sense and stereognostic sense was normal.

The deep reflexes were impaired but present, plantar reflexes were normal. Intelligence ppeared t be normal. EEG N definite pathological findings. Paper chromatography of the urine: Normal amino acid pattern. Immunologic examination revealed lack of γ-globulin.

Case 4

T. M., born 1957. This boy was 6 years and 2 months old when admitted. The maternal grandfather had dilated veins in the ears, the scalp and under the eyes, but was healthy. Pregnancy and delivery had been uneventful. Birth weight was 2275 g. There were no neonatal complication and psychomotor development during the first year of life was normal. The patient talked at the usual age and started to walk at 14 months of age. At 17–18 months the gait was strikingly ataxic and became more so with increasing age. At 5 years of age the parents noticed that his eyes were red. He had had bronchitis every winter and once pneumonia.

Findings. On examination the length was 116.5 cm and the weight 17.5 kg. Telangiectasias were present in the bulbar conjunctivae, under the eyes, in the ears, on the dorsal side of the ears, over the mastoid process and in the scalp. The skin on both temples and in front of the ears was poorly pigmented and the hair was thin. He presented nystagmoid eye movements, turned his eyes upwards when focusing and had a convergent strabismus on the right eye, a fixed facial expression and spoke indistinctly. The head tended t fall forwards. He had a stooping carriage when walking and the gait was taxic with the legs widely apart but he walked without support. He had choreoathetotic movements of the face and upper extremities, but no spasticity or rigidity. The co-ordination was uncertain and Romberg' test positive. Dysdiadochokinesia was present and the movements were slow. Deep reflexes and plantar reflexes were normal. The intelligence appeared to be normal. Paper chromatography of the urine: Normal amino acid pattern. Immunologic examination revealed lack of γ-globulin. Immunologic examination of the grandfather's serum showed the normal amount of γ-globulin.

Case 5

A. P S., born 1956. This boy was 8 years and 2 *months old when admitted.* H was an adopted child. The mother had had epilepsy during pregnancy No other information was available concerning the child s family or birth. He came to the foster parents at 4 months of age. Motor development was moderately retarded during the first year of *life*, and possibly more so during the second and third years of life. He walked alone at 3 years of age. The eyes had been red from about 7 years of age The patient had had sinusitis and bronchitis several times and during the last 6 months he had had frequent outbreaks of facial erysipelas. His intelligence was below normal and his progress during the last two years had been less than the expected.

Findings. On admission the patient was 12 cm below the average height for his age. Telangiectasiae were present in the bulbar conjunctivae, especially in the left eye, and also in the auricles. There was no nystagmus or strabismus. He spoke slowly and the facial expression was rather immobile. He walked unsteadily with the legs widely apart and presented athetoid movements of the head, neck and hands but no spasticity or rigidity The co-ordination was uncertain and Romberg s test positive. The movements were retarded and slow The deep reflexes were symmetrically impaired, the plantar reflexes equivocal. EEG showed spike focus in the left parietal temporal region. Examination by a psychologist in 1962 and 1964: "Leiter I.Q 81 and 70 respectively The difference cannot be given prognostic significance." Immunologic examination revealed about 10 per cent of the normal amount of γ_A-globulin.

Summary

Five cases of ataxia-telangiectasia are reported. In 4 cases, there is no parental consanguinity In the other case the parents are *unknown. The maternal* grandfather of one of the patients had telangiectasiae in the scalp, face and ears, but no ataxia and the γ_A globulin content *in the serum was normal.* There was no known case of ataxia in any of the families. All of the patients had had bronchitis, *but only 3 of them had been particularly* susceptible to respiratory infections. The growth was retarded in all. None of them presented deformities of the feet or kyphoscoliosis.

Only in one patient was the motor development retarded during the first year of life, in the remaining the disturbance of the psychomotor development appeared in the form of ataxia in the course of the second or third year of life when the patients started to walk.

One patient developed telangiectasiae at 2–3 years of age, the others at 5–7 years of age. Patches of poor pigmentation of the skin in the face were present in 4 patients. Choreoathetotic movements and a tendency to turn the eyes upwards when focusing was observed in 4 cases.

The sensibility was normal in all cases. The deep reflexes were normal in one case and impaired in four In the latter normal reflexes had been demonstrated at an earlier age

Intelligence fell within the lower limits of normality in all patients. No definite progressive impairment was demonstrable

The EEG was normal in _ cases. In one patient a moderate general cerebral dysrhythmia was found, most marked over the right temporal region but no epileptogenic activity In another a focus was registered in the left temporal region. One patient had not been submitted to EEG

Paper chromatography of the urine revealed a normal amino acid pattern in 4 cases, the 5th was not examined.

Immunologic examination of the patients sera revealed lack of γ_A-globulin in 4 cases and a reduction to about 10 per cent of the normal amount in one

Acknowledgement

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Addendum

Case 4 T.M. born 1957 died of lymphosarcoma veli palatini in the autumn 1965

References

1 Boder, E. and Sedgwick, R. P Ataxia-telangiectasia. *Little Club Clinics in Developmental Medicine, 8.* 110, 1963.
2. Bonnevier, J O A case of ataxia-teleangiectasia. *Acta Paediat Scand, 50* 283, 1961
3. Brandt, S.: Cefalo-oculo-cutane telangiectasier (Louis-Bar syndrom). Progressiv taxi, demens, chronisk bronchitis og okulaere forandringer hos en 13-årig dreng *Nord Med, 62.* 1819 1959
4. Fireman P Boesman M. and Gitlin D.: Ataxia telangiectasia, dysgammaglobulinaemia with deficient gamma$_1$A(beta$_2$A)-globulin. *Lancet, II* 1193, 1964.
5. Hammer E.: Ataxia-telangiectasia. *Acta Neurol Scand, 38.* 188 1962
6. Harboe, M.: Immunologiske abnormiteter ved ataxia-telangiectasia. *Vitenskapelige arbeider fra Sentralinstitutet for cerebral parese,* Oslo, *I.* 94, 1964.
7 Louis-Bar, D.: Sur un syndrome progressif comprenant des télangiectasies capillaires cutanées et conjonctivales symétriques, à disposition naevoïde et des troubles cérébelleux. *Confin Neurol 4* 32, 1941
8. Peterson, R. D A., Kelly W D. and Good, R. A.: Ataxia-telangiectasia, its association with a defective thymus, immunological-deficiency disease, and malignancy *Lancet I* 1189 1964.

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The Danish Pediatric Society

Meeting April 7 1965

J Vesterdal Referral of Children to Child-Psychiatric and Psychological Treatment

During the period 1.IX.1957–1.I.1965 a total of 7264 patients were admitted to the Pediatric Department in Sundby Hospital and 570 of these were examined by the psychologist attached to the department In 68 cases, only intelligence tests were carried out and, in the remainder projective tests, conversations with the parents etc. were carried out in addition. In the majority of these cases, the parents were given advice, recommendations about schools, nursery schools etc. and, in 129 cases a change in environment in the form of a stay in the country for some months in special children's homes etc. was arranged.

Forty-one of the children exhibited psychic disturbances of such an extent that psychotherapy was considered necessary for the children or the mothers. Four of these children were referred to a colony for observation or home for treatment. The remaining 37 and it is this fraction that we are interested in were referred to various child psychiatric or psychological clinics in Copenhagen.

The question now arises: How many of these 37 reported for treatment and adhered to it to the extent which the therapist considered necessary?

It has been demonstrated that one child is still on a waiting list and that in three cases, during the intervening period other measures have been undertaken (placement in approved schools etc.) Out of the remaining 33, eight simply did not report when summoned for admission or out-patient treatment as the mothers were not interested in continuing Fifteen commenced out-patient treatment but ten of them stopped after one or a few sessions and only five completed the treatment.

Thus, out of 33 patients, only 12 completed the treatment. The results appear to be better for patients admitted than for out-patients. The length of the period of waiting, strangely enough, did not appear to have any definite influence upon the result

Many reasons for these poor results may be considered the primary reason being that the mother is unable to come because she goes out to work or that she sees no reason for psychotherapy e.g. because she is not interested in the child or does not understand that the psychosomatic symptoms can have a psychological cause.

These figures speak for the provision of more beds in child psychiatric departments, as it is easier to maintain contact with the mother when the child is admitted. Group therapy for the mothers in the evenings or establishing contact between the mother and the child psychiatrist while the child is in the pediatric department would probably improve conditions and measures such as these have already been instituted

DISCUSSION

P Plum: One of the reasons that treatment is not carried out must be the long period of waiting. Pediatric departments should maintain contact with the parents during the period of waiting.

J Lenstrup: The correct solution is probably to establish psychiatric psychological teams attached to pediatric departments.

P W Braestrup In the Pediatric Department in the Copenhagen County Hospital

in Gentofte, we ha e had correspondingly poor experience in carrying out of psychotherapy It is considered that lack of motivation on the part of the mothers is the main cause of this. Pediatric Department must probably make greater efforts to get the mothers interested before they refer the children for psychotherapy

O Steinicke. The clinics concerned probably do not d enough to get hold of the children and this is probably due to the long waiting lists.

P Plum. The parents (mothers), naturally enough, are unwilling t co-operate with a new therapist after they have previously had contact with a doctor in the pediatric department.

H Andersen. This account should be extended to include more children.

Emilie Faber The defaulters belong to families whose environment is not a reason for treatment.

S. Vestermark: Panniculitis Nodularis

A case of spontaneous panniculitis nodularis (Weber-Christian syndrome) is reported in a newly born girl. The liver was enlarged and the ESR raised but no pyrexia was present. The nodules disappeared in the course of pproximately six weeks without leaving scars. On biopsy phagocytic histiocytes, giant-cells, lymphocytes and granulocytes were demonstrated in the subcutaneous tissue.

K. E. Petersen: Congenital Agranulocytosis

A fatal case of agranulocytosis is reported in boy who died t the age of six months. N immune mechanism or hereditary origin could be demonstrated.

Since birth, the child had exhibited a tendency t skin infections and thrush. He was admitted at the age of three months and during the st y in hospital he suffered from recurrent ot tis and pulmonary infiltrations In addition t conjunctivitis and more or less constant thrush. As hypogammaglobulinaemia was demonstrated, the infant was treated with injections of γ-globulin without any effect being observed on the serum concentration. The peripheral blood smear contained only few mature neutrophile granulocytes. The bone-marrow also showed signs of agranulocytosis with only 2% neutrophiles, with segmented nuclei and marked displacement to the left of myelopoiesis. Concomitant with commencing steroid therapy an increase in the number of neutrophiles occurred (up to 29% in the peripheral blood) and clinical improvement, but the condition deteriorated again and the case ended fatally probably from septicaemia or a generalized fungus infection. Permission for autopsy was not obtained.

The etiology and pathogenesis of the condition are discussed Cyclic agranulocytosis and chronic benign granulocytopenia can be excluded by the course of the disease. Neutropenia provoked by sulphonamides is possible as a sulphonamide preparation was administered shortly before admission and another was administered for a brief period during the stay in hospital. As the condition improved and then deteriorated further without further sulphonamide administration, this possibility is not considered probable. Leucocyt gglutinins were not demonstrated in the child serum. In 1956, Kostmann described the syndrome "hereditary infantile agranulocytosis" with a simple recessive heredity Since then, pproximately 20 cases of congenital neutropenia have been described. It has only rarely proved possible to demonstrat familial incidence. Familial incidence could not be demonstrated in the case reported here and it must be classified in this category f neutropenias in childhood.

DISCUSSION

P W Brandrup mentioned a child with complet agranulocytosis. This child died at the age of six years.

P Plum emphasized that valuable information may be obtained by counting the cells in the bone-marrow and by determination of the proportion between the red and whit precursors.

S Vestermark M tabolic Acidosis and Pulmonary Hypertension

A girl aged almost two years exhibited at the ge of ten months, a total of four nocturnal episodes of cyanosis, foaming at the mouth loss of consciousness, grunting respiration and fibrillations around the mouth. She had still a tendency to cold, slightly cyanotic extremities although this was now less pronounced than previously The EEG was normal. During the entire period of observation, the child had slight metabolic acidosis with the following average alues: pH 7 28, pCO 40.2 mm Hg, base excess —7.3mEq/l and standard bicarbonat 18.3 mEq/l. Further slightly raised lactic acid levels in the serum were demonstrated (1.92–3 7 mM/l) and the GO transaminase was raised (2.6–3.7 mM/l). The pyruvic acid level in the serum was normal. ECG showed right-sided entricular hypertrophy and selective angiocardiography with mea surement of pressure revealed pulmonary hypertension (87/30 mm Hg in the pulmonary artery) The oxygen saturation was 79 per cent. In addition, a small patent ductus arteriosus with a left-right shunt was demonstrated. The clinical condition had im proved in the course of the past year The cause of the metabolic acidosis and the hyperlactataemia is not known. The cyanosis was not sufficient to explain it and nu merous investigations with the object of demonstrating possible errors in the inter mediate metabolism revealed normal values.

DISCUSSION

The pathogenesis and diagnosis were discussed by *B Friis-H nsen, H Andersen J I ausdal, N Hobolth P W Brandrup* and *P Kildeberg*

P Kildeberg In this patient, the history is dominated by the tendency to cyanosis and the pulmonary circulation was demon strated to be defective with pulmonary hypertension. It seems reasonable to presume that the very slight chronic metabolic aci dosis was due to accumulation of lactic acid The serum lactate values were definitely raised and the discrepancy between the numerical serum lactate value and the base excess values are *not* necessarily evi dence against pure lactic acid acidosis as lactate ions may be lost in the urine. Other authors including Israels *et al.* (*Pediatrics* 1964) have described lactic acid acidosis as a result of a congenital enzyme defect, but could not the circulatory disturbances with tissue hypoxia be the most reasonable ex planation in this patient?

P A. Krasilnikoff: Myelofibrosis

Myelofibrosis has been described on nu merous occasions in adults but only in 22 cases in children and, of these, 15 belong to the so-called primary form, i.e. without known etiology and the remainder belong to the secondary form, all of them being associated with leukaemia.

In the Pediatric Department of Sundby Hospital, a boy of nearly four years was admitted with myelofibrosis. H had al ways" shown a tendency to bruising The li er and the spleen reached 1 2 finger breadths below the costal margins and there were subcutaneous haemorrhages on the ex tremities. The Hb was 10.1 g/100 ml and the thrombocyte count 47 000 per mm but, at the commencement, the white blood pi ture was normal. The bone-marrow showed few megakaryocytes but was otherwise of normal composition. After the condition had remained stationary for eight months, the Hb fell to 6.5 g/100 ml and the thrombocyt count to 13,000 There were numerous cu taneous haemorrhages, the spleen reached the iliac crest and the bone-marrow was hyperplastic w th few megakaryocytes. Essential thrombocytopenia was suspected and splenectomy performed. The spleen weighed 250 g and was found to be microscopically normal. After two further months, the Hb and thrombocyte count fell even more and numerous transfusions were required. A great percentage of immature red precursors were present in the peripheral blood and the total number of nucleated cells was 30,000. The bone-marrow was hypoplastic

with marked erythropoiesis and a megaloblastic tendency

The diagnosis of myelofibrosis was then established and on biopsy from the iliac crest the marrow was found to be of low cell content with signs of previous haemorrhages, necrosi and formation of fibrosis hich were considered to support the diagnosis. Treatment was commenced with vitamin B_{12} which did not produce any effect on the megaloblastic blood picture. Decadurabolin was then employed and later supplemented with prednisone and numerous transfusions. The liver enlarged rapidly and gradually reached the level of the umbilicus. The total number of nucleated cells in the peripheral blood increased violently to a maximum of 223,000 and of those between 16 and 77 per cent were red precursors, 1–2% blast-like cells and the remainder were mature leucocytes and myelocytes. The patient general condition gradually deteriorated and he died at the age of 3½ years.

At autopsy the marrow in the femur was found to be maximally hyperplastic and dominated by immature cells from myelopoiesis. Occasional erythroblasts were present, no megakaryocytes and no signs of myelofibrosis. In the liver periportal infiltration with myeloid cells was found to extend out into the sinusoids and occasional erythroblasts but no megakaryocytes. In the kidneys and lungs also, myeloid (leukaemic) infiltrations were found.

This case thus presented, at the beginning of the illness, as myelofibrosis as was suggested by the pronounced erythroblastosis and the pernicious anaemia-like blood picture the chronic course and the changes in the biopsy from the iliac crest. However autopsy revealed a myeloid leukaemia as suggested by the myeloid infiltrations in the liver lungs and kidneys. In particular the changes in the liver serve to differentiate the case from myelofibrosis where myeloid met plasia is primarily encountered in the sinusoids while, in the periportal spaces, as in every li er infiltrations with lymphocytes and plasma cells re observed. It is impossible to stat whether the disease commenced as a leukaemia. The extremely high total number of nucleated cells in the peripheral blood with varying distribution between the red and white precursors and the maximally hyperplastic bone marrow cannot, with certainty be taken as evidence of either of the possibilities. The other clinical symptoms do not permit any differentiation.

In his thesis on myelofibrosis in 1958, Pinholt Andreasen included a material of five children of whom four probably suffered from lymphatic leukaemia with bone-marrow changes which were impossible to differentiate from myelofibrosis.H concluded that, in certain cases of myelofibrosis, the condition seems to be part of a leukaemia, mainly lymphatic leukaemia but it seems reasonable t presume that this phenomenon can also occur in myelogenic leukaemias. The case reported here could fit in with the latter presumption.

DISCUSSION

The diagnosis was discussed by *P Plum* and *T Iversen*.

J From and S. Vestermark. Familial Hypercholesterolemia

A girl aged five months on the first admission was admitted with severe bilateral pneumonia, and was treated with various antibiotics. N definite improvement occurred until antibiotic therapy had been continued for fiv weeks. During the entire course of the disease, the stethoscopic and radiographic findings were negligible compared with the poor clinical condition. The child was the fifth of five siblings and there was no known predisposition to hereditary defects.

On account of the course of the disease, special interest was taken in possible congenital defects. Immuno-electrophoresis of serum proteins showed extremely high values for alpha-2 lipoprotein and alpha-2-macroglobulin and this led to investigation of the serum lipids. In the patient her father and an older sister raised serum cholesterol and phospholipid values were found. N evidence

of nephrosis, hypothyroidism, diabetes or liver disease was found.

Familial hypercholesterolemia is a relatively frequent condition in adults but it has rarely been described in children. As the condition is, by and large, symptomless in childhood, it is only found by examining children in families with known hypercholesterolemia. Fatal cases have, however been described in children with hypercholesterolemia e.g. on account of a xanthoma in a coronary artery.

Various forms of treatment have been described: reduced administration of dietary cholesterol by substitution of the fatty acids in the diet with unsaturated fatty acids and treatment with nicotinic acid and thyroxin analogues. However all forms of treatment have only temporary effects. This patient was treated with birodan (thyropropicic acid) for 14 days after which the serum cholesterol fell to normal values. On the other hand, no effect was observed from a diet with a high unsaturated fatty acid content for 14 days.

Meeting May 21 1965 with the Danish Tuberculosis Association

Nicholas Matsaniotis (Athens): BCG Fatalities; New Aspects on the Etiology

J. Seltoft. Follow up Examination of 93 Cases of Tuberculous Meningitis after the Introduction of Streptomycin Therapy

N. Matsaniotis. On the Histopathology and Immunology of the Tuberculin Reaction

O. Horwitz: Training Nurses to Read Tuberculin Reactions

Torben Iversen

The Finnish Pediatric Society

Meeting October 12, 1963

T. Peltonen: Protein Bound Iodine in Blood in the Newborn period

One method of assessing thyroid function is to determine the amount of hormone produced by the gland. This is measured by the protein bound iodine (PBI) which, however includes some inactive iodine compounds. The values are variable during infancy and show considerably higher figures during the first trimenon of the first year of life than later. However during the first 24 hours very low figures may also be found. These low values are normalized already on the next day. In 72 exchange transfusions the high PBI of 54 newborns dropped to the adult level, but in a few hours the infantile organism restored them to their former level.

H. Salmi. The Physiology of Vitamin B_{12} during Foetal Development

Vitamin B_{12} induces the synthesis of desoxyribonucleic acid and probably ribonucleic acid as well. Further it has an effect on protein metabolism through its influence upon the synthesis of methionine. Thus, vitamin B_{12} plays an important role in the growing organism.

According to the literature it is evident that the foetus pursues a parasitic action vis-à-vis the mother both in man and in animals. This has been established in many different ways: The serum B_{12} level of pregnant women decreases with the advance of pregnancy; the tissue concentrations are decreased during pregnancy in test animals,

the serum level of the child t the moment of birth is higher compared to the mother the absorption of the itamin is increased and the urinary excretion is lowered in preg nant women etc. Further previous literature shows that severe vitamin B_{12} deficiency in the foetal period causes a multitude of developmental disturbances, especially mal formations of the nervous system.

In the present study the tissue vitamin B_1 concentrations in human and animal foetal tissues were measured. In human foetuses the greatest concentrations were found in the kidney adrenal glands and liver The thymus, spleen, brain and bone marrow had the lowest level. No correlation was established between the length of the foetus, i.e. *the stage of development and the vitamin* B_{12} concentration during human foetal development. With the advance of preg nancy a declining trend was found in the brain tissue. A definite concordance was noted between the values obtained for the different tissues of the same foetus, i.e. in each foetus the concentrations were throughout of a high or low level. Rat foetal tissues showed results consistent with human tissues. The role of vitamin B_{12} in the various foetal tissues and the foetus-mother balance of the vitamin was discussed.

The foetal uptake of radiovitamin inject ed into the maternal rat was studied by autoradiographic methods. The stripping film technique was employed t establish the localisation at the cellular level. Kidney tissue showed the most interesting results the activity there being localised in the outer part of the cortex in the proximal convoluted tubuli around the glomeruli. The possible role of vitamin B in the metabolism of the proximal tubuli and in the production of erythropoetin was discussed

P Villa: A Rare Case f Enterocystoma with Peptic Ulceration and R troperitoneal Perforation

The term enterocystoma is used here to differentiat the lesion described below from the more common duplications which are more or less connected w th the normal alimentary canal of the patient. The retroperitoneal location of enterocystoma with no connection to the gut or mesentery is a very rate entity and the clinical picture presented by the lesion is thus a most confusing one.

A girl with normal birth and early infancy became anemic and restless at three months of age Her weight failed to increase and she had occasional vomiting and pparent attacks of abdominal pain. Except the hypochromic anemia and moderately elevated ESR she had no abnormality in laboratory tests. The anemia did not respond to any form of treatment, and in adequate hemoglobin level was restored only by blood transfusions. The X ray of the heart and *lungs was normal. On i.v pyelography the* urinary tract was normal, only the lower pole of the left kidney was thought to be slightly expanded.

At seven months of age a diffuse mass was palpated in the left upper abdomen. Urotomography was normal, and the renal an giography did not reveal anything pathological. The pararenal space was aspirated and incised, and brownish dark pus was btained. The bacteriological smears as well as culture of the pus were negative. At the lower pole of the spleen there was an indefinite mass, barely palpable. At this stage the blood pressure was elevated, and the excretion of vanillin mandelic acid was slightly above normal.

At laparotomy at eight months of age a retroperitoneal tumor of the size of a ping pong ball was found, closely attached to the tail of the pancreas as well as to the spleen which both had to be removed with the tumor The postoperative course was uneventful, the child recovered from the anemia, and the gain of weight has been normal during the three months of follow-up.

The tumor was a thick walled cyst which contained all the layers of the alimentary canal and was lined with hypertrophic gastric (pyloric) mucosa. A peptic ulcer with perforation was found and the neighbour ing parts of spleen and pancreas showed microscopic signs of ulceration and hemorrhage.

I Välimäki: The Physical Working Capacity of Diabetic Children

The bicycle ergometer method of Sjöstrand was used f r the determination of the working capacity of 119 healthy and 76 diabetic children, ranging in age from 6 to 16 years. The studies were performed during the autumn of 1960 and 1962 in the Cardiorespiratory Research Unit of the University of Turku, Finland. In both groups the working capacity correlated well with age and the logarithm of weight height, surface area, and vital capacity Insulin dose and duration of the disease gave poor correlations. In the group of smaller individuals the healthy boys possessed greater working capacities than the diabetic ones. Older diabetics reached the level of the healthy boys. Working capacities were the same among the younger healthy and diabetic girls. Differences between the groups became increasingly evident in older and larger girls.

Meeting November 27 1963

H Stutte (Marburg an der Lahn) Psychopathology of Endocrine Disturbances in Childhood

Meeting December 5 1963

J Saukkonen. Molecular Diseases

H Nevanlinna Aspects of Human Genetics

K. Launiala. Saccharose Intolerance

Meeting February 8 1964

E. B Nordlund (Stockholm): Child Psychiatric Aspects on the Treatm nt f Chronically Ill Children and their Parents

The child s experience of chronic illness differs from that of an adult. The younger a child is, the more parents have to help to an atmosphere which allows the child to develop in a fairly normal way in spite of the illness. When the child grows older it successi ely must take over responsibilities and finally has to become more or less independent depending on the kind of illness. Different illnesses create different problems which can impair the child s development. Illnesses with a bad prognosis involve serious problems for both patient and parents.

The child s experience of death at different ages is important. Puberty has a strong impact even on healthy children when life seems meaningless to them, for the chronically ill child it can be a disaster As t treatment, the most important thing for the child and the family is to have the same doctor all the time.

Meeting May 8 1964

T Putkonen. Sarcoidosis in two Children and in the M ther of ne of them

"Sarcoidosis is a systemic granulomatous disease of undetermined etiology and pathogenesis. Mediastinal and peripheral lymph nodes, lungs, liver spleen, skin, eyes, phalangeal bones and parotid glands are most often involved, but other organs or tissues may be affected. The Kveim reaction is frequently positive and tuberculine type hypersensitivities are frequently depressed Thus begins the description of this disease as accepted at the congress in Washington in 1960. The reported frequencies of the various manifestations of the disease ary greatly depending on what speciality the collectors of a material represent. Up to a few years ago the patients seen at the Helsinki Univ ersity Dermatologic Clinic were almost

entirely chronic cases referred because of the skin manifestations of the disease. Since then the material has changed considerably as hospitals of other specialities began sending us patients for the performance of the Kveim reaction.

As elsewhere, children have formed only a small minority in our material. Of our 87 patients with sarcoidosis only two are children. The first one was sent to us at the age of 9 years 4 months. The right parotid gland of this boy had been swollen for 8 months, and 5 months after onset a biopsy had given the diagnosis sarcoidosis. On admission he was febrile and had, in addition to an indolent parotitis, a slight facial paresis on the same side and a uveitis on both sides. Thus a diagnosis of uveoparotid fever was made. During the preceding 4 months a reddish-brown tumor had developed in the skin under the left corner of the mouth; the histological structure was that of sarcoidosis. Another subcutaneous tumor 3 7 cm large, had appeared within 2 months on the upper part of the left arm and biopsy showed sarcoidosis of the deltoid muscle. Chest roentgenograms disclosed some small mottled opacities in the lungs and enlarged lymph nodes were palpable on the neck and in the axillae. While in hospital he developed a talcum granuloma in the scar of the muscle-excision and this too had the structure of sarcoidosis. The Mantoux test with 1000 TU of OT was negative as was the Kveim reaction.

The other child was 6 years 1 month old girl who had had intermittent fever cough and dyspnoea during the preceding 2 years. Her chest X-ray showed enlarged hilar shadows on both sides and miliar opacities in both lungs. Biopsies from lymph node on the neck and from the considerably enlarged liver proved to be epitheloid cell granulomas. The Mantoux-test with 1000 TU of OT and PPD was negative though the patient had been successfully BCG vaccinated in 1957. The Kveim reaction was negative.

In spite of their negative Kveim reactions both patients fulfilled the requirements for a diagnosis of sarcoidosis as stated by the Washington congress, "consistent clinical features, together with biopsy evidence of epitheloid tubercles or a positive Kveim test. An additional feature of interest in the latter case was that her mother too had sarcoidosis with a positive Kveim reaction.

S. van Creveld (Amsterdam): Longlasting Experience in Glycogen Disease

Meeting August 26–29 1964

J. Lorber (Sheffield): Present Outlook for Children with Hydrocephalus

B. D. Corner (Bristol): A follow up Study of Children Presenting with Abnormal Neurological Signs in the First Week

One hundred babies of birth weight over 5 lbs 8 ozs, who as a result of severe asphyxia or traumatic brain damage were carefully examined on the third and fifth days and have been followed up for a period of at least two years. The degree of asphyxia was graded.

R. L. Takkunen and M. Frisk. Follow up Examination of 110 Small Prematures at the Age of 6–7 Years

For the purpose of this study small prematures were defined as prematures with a birthweight of 1750 g or less. The material consisted of all those small prematures treated at the Helsinki University Children Hospital in 1955–1957 whose parents could be induced to bring them for a follow-up examination. This was performed during the first half of the year in which the children reached the age of 7 which is when compulsory education begins in Finland.

The purpose of the study was to assess the physical and mental development as well as the maturity of these small prematures. The examination programme consisted of a week's observation on a ward, general medical, anthropometric, neurological, psychiatric and psychological examination as well as EEG radiological, laboratory ophthalmological and otological consultations.

The total number of small prematures treated originally during the period in question was 280. A primary mortality of 27.9% reduced the number to 202. Of these 110 (54.5%) were brought for examination. 33 of them were twins.

The mean height and weight of these prematures were below those of term children at this age. The mean deviation was 2 cm in height and 1 kg in weight. 14% of the children were under the 10th percentile limit as to height and 18% as to weight.

In 41% of all the children examined definite neurological defects were found: 12% with various paretic conditions and 29% with functional neurological immaturities. The twins were less often affected: in only 24% as to 49% in the others.

The mean intellectual level, determined by the Terman–Merrill test as standardized Finland, corresponded to an IQ of 94.8. is well below the present normal mean of about 105. Lowering of the mean was due mainly to a relatively high frequency of backward children (IQ 75–90) and a few cases with a grave defect in intelligence.

In 54% symptoms were noted, which were regarded as neurotic. In this no difference was found between the twins and singletons. The remaining 46% included some cases of psychoinfantilism.

School maturity was assessed taking into account possible physical, psychic and social handicaps. 49% were assessed as immature for ordinary school.

G. A. Neligan (Newcastle). Neonatal Hypoglycaemia and Intrauterine Nutrition

The published observation that babies of low birth weight relative to their maturity have significantly low postnatal blood sugar levels is being extended:

(*a*) The postnatal changes in the blood sugar levels are being followed very closely for 48 hours, relative to antenatal and intranatal factors, and to postnatal stress, temperature, blood pressure and feeding. Over 50 babies should have been studied by the late summer.

(*b*) The later development of babies who had significantly low postnatal blood sugar levels is being followed. Some children should have been followed to the age of 4½ years by the late summer.

K. Keuralainen. The Immunological Significance of Hassal's Corpuscles in the Thymus (A Histochemical Study)

The important role of the thymus in the immunological defence mechanisms of the organism has been well established during the last few years. The function of Hassal's corpuscles—the peculiar whorl-like microorgans in the thymic medulla—is however still obscure.

Cell breakdown and occurrence of so-called chromatin particles are very often seen in Hassal's bodies of thymic sections stained with hematoxylin and eosin. When demonstrating histochemically certain enzymes in the human thymus, it was found that many Hassal's corpuscles contain 5-nucleotidase, an enzyme which participates in the breakdown of nucleic acids, but this enzyme was lacking outside of these structures.

By using fluorescent antibodies it was shown also that γ-globulin containing cells are destroyed in Hassal's bodies and such cells are seen also in the thymic medulla and cortex.

It is suggested that 'forbidden clones' are liminated in thymic Hassal corpuscles or/and some important nucleic material from leucocytes and thymic cells is liberated there for reutilization.

J. Visakorpi: Laboratory Tests Used in the Diagnosis of the Malabsorption Syndrome

The material studied comprises 105 children, most of them infants, suffering

from different gastrointestinal disorders such as celiac disease chronic enteritis and *secondary malabsorption syndromes of va nous etiology* The material included also as control group of chronically ill children without gastrointestinal symptoms.

Intestinal absorption was studied with a 3-days fat balance the Lipiodol-test, the Xylose excretion test, the peroral glucose loading test and by determination of urinary FiGlu.

The results were correlated with each other and with clinical diagnosis and duodeno-jejunal histology

J. A. Black (Sheffield): Recurrent Haematuria in Childhood

A clinical and pathological study was undertaken of 46 cases of recurrent haematuria using renal biopsy The cases could be divided into three groups: those without a definite illness at the onset those with an onset with an attack of Henoch-Schonlein purpura, and those with an onset resembling acute nephritis.

In the first group the attacks of haematuria were provoked either by exercise or by minor upper respiratory infections. The results showed that in the majority of cases the histological diagnosis was one of local nephritis, but the lesions tended to be more widespread in the Henoch-Schonlein group

C. J. Hodson (London) Chronic Pyelonephritis in Children

Serial intravenous pyelograms provide the only reliable means of determining whether chronic pyelonephritic scarring is occurring or increasing in children with urinary infections.

The paper is based on experience in this field over a period of ten years. It outlines the unexpectedly high incidence of pyelonephritic scarring in the child population and illustrates how the early lesion can be detected, the course of the disease followed and the effectiveness of any form of therapy checked.

The association of vre reno-ureteric reflux with this condit stressed once again.

M. MacGregor (Warwickshire): Clinical Aspects of Persistent Urinary Infection

This paper describes a thirteen year follow-up of 82 children treated in a paediatric unit for acute urinary infection.

The main conclusions are

1. More than a quarter are still infected.

2. Cysto-urethrograms show that many of them have correctable urological bnormalities.

3. A wet film technique of urine cell counting is shown to be a reliable screening method, applicable to wards and outpatients, as well as to school medical clinics and doctors surgeries.

Illustrative slides are shown.

B. Landtman. Epidemiological Aspects of Congenital Heart Disease

From 1950 to 1963, approximately 25,000 children were referred from different parts of Finland for cardiac studies to the Cardiac Clinic of the Children Hospital in Helsinki.

The series comprised over 3000 cases of congenital heart disease The incidence of these defects ppears to be pproximately the same in different parts of the country

From 1948 to 1963, 4172 autopsies were performed at the Children Hospital. The incidence of congenital heart disease in these series has gradually increased from 4.8 to 16.8%.

The incidence of acut infectious diseases in Finland is usually highest in November–February Children with congenital heart disease were more often born in the late summer and early atumn—8 to 9 months after the peak of infectious diseases—than normal children.

Children with congenital heart disease were comparatively often first born children.

H. Hahla Health Services in Finland

Maternity Health Services. The law of 1944 provides that there must be at least one

maternity and one child health centre in each commune.

The MH services include consultations by physician and midwife and home visits by the latter. A comprehensive psychoprophylactic relaxation course is included. The number of centres today is 3100. The number of midwives serving them is 1038.

In 1963 the number of births was 82 417 (birth rate 18.1) and 95.9% of all mothers went to maternity hospitals for delivery. The maternal mortality rate in 1963 was 0.50.

Child Health Services. The child health centres serve children in the age group 0–7 years. The services are regulated by the same law as maternal health services. The CH services consist of consultations by physician and public health nurse and home visits by the latter. Usually the health supervision of the new born (2 weeks) is done by the midwife, who then turns the infant over to the PHN.

The number of CH centres today is 4200. The number of public health nurses serving them is 1483.

The infant mortality rate in 1963 was 18.0.

School Health Services. Medical and general [illegible] are provided by law (1944, 1952, 1956) for all elementary school children. The health services for secondary school children are carried out according to separate law and are also subsidized by the state to about 75 per cent. According to the law of 1952 there must be one school physician in every community. The public health nurses are requested to act as schoolnurses as well. The number of elementary school children in 1963 was 596.000 and that of secondary school children 240 000. The doctors' examinations are generally carried out upon commencing, and leaving school and once during intermediate years. Those pupils receiving vocational guidance undergo a special examination by the school physician around the age of 15. Whenever the teacher, PHN or doctor think an extra examination necessary the pupil is directed to the school physician or a specialist.

M. Pulkkinen: Detoxication Mechanisms in the Fetus and Newborn Child

In addition to its pharmacological significance, detoxication has an important function in physiological metabolic processes. The conjugation of bilirubin to glucuronide or sulphate is a very important reaction in the newborn child. The same applies to steroid conjugation. The acetylation reaction is less well known than the glucuronylation and sulfurylation reactions. The conjugation of different substances with β-D-glucosiduronic acid is weak in the fetus and even postnatally. This was established first by Hartiala & Pulkkinen in 1955. The rate of glucuronylation is maximal in the rat at the age of one month, and hence before sexual maturity. Prolonged administration of estrogens or androgens lowers the rate of conjugation in a young animal to the level found in adult animals. It may be of value to study this question also in humans because of its potential bearing on the dosage of drugs in pediatric practice. It may also be advisable to reconsider the indications for the administration of steroids.

Sulfate conjugation is fairly well developed already in the fetal period, but continues to develop as the newborn grows into an adult. The activities of the enzymes, such as β-glucuronidase and the various sulphatases that hydrolyze the conjugates also increase from birth to maturity but the increase does not parallel that of the enzymes which promote conjugation.

When the treatment of icterus of the newborn is considered in the light of the above influences the attempts should be made to equilibrium between conjugated and unconjugated bilirubin in favor of the former in order to promote its excretion. Conjugation should be accelerated or hydrolysis inhibited. These possibilities have already been tested in clinical practice.

T. Peltonen and L. Hirvonen. Heamodynamic Changes during the Perinatal and Neonatal Periods

X-ray findings during the first breath. During the passive phase the elasticity of

the thorax causes the filling of the upper airways with air. The essential aeration, however is always due to the active respiratory work of the infant. The diaphragm plays the most important role in the respiratory effort.

Ductus arteriosus. Before the first breath radio-opaque material injected into the superficial jugular vein enables the visualization of the "via dextra" right atrium and ventricle, pulmonary artery, arterial duct and aorta. After the aeration of the lungs the dye entering the right heart shows the pulmonary arteries, but no longer the aorta. The left pulmonary artery shows with each cardiac cycle the weakening of the contrast caused by the dye-free blood flowing from the aorta. Under the same conditions arteriography demonstrates the thread like arterial duct with flow from the aorta into the pulmonary arteries. The flow increases in hypoxia.

Before the aeration the blood pressure in the pulmonary artery is somewhat higher than that in the aorta. When respiration starts the aortic pressure remains unchanged, but the pulmonary arterial pressure decreases. This reverses the flow in the ductus. Increased oxygen tension decreases the resistance of the pulmonary circulation—conversely hypoxia increases the resistance and pressure. The high pulmonary pressure disturbs pulmonary function. The neonate resists the development of a vicious circle by letting oxygen-saturated aortic blood flow via the ductus into the pulmonary vessels when asphyxia threatens.

Foramen ovale. There is a muscular valve which regulates the flow from the inferior caval vein into the left atrium. The flow via the foramen ovale practically terminates when the flow through the lungs into the left heart is large enough. Clamping of the cord before the first breath causes a lack of blood in the left heart and the heart size diminishes considerably.

Insufflation of the lungs. Insufflation with gas opens the alveoli and sucks blood into the small arteries and capillaries. The insufflation pressure does not exceed 20 mm Hg. A higher pressure increases the right ventricle pressure and later the aortic pressure decreases. The haemodynamic changes were demonstrated with microscopic films.

Meeting October 17 1964

E. K. Ahvenainen. The Causes of Death of Premature Infants

There is a trend toward individual diagnosis of the disease of a premature infant. For this reason also autopsies of these patients should be used to verify the clinical diagnosis more than has been customary. Hyaline membranes are the commonest autopsy finding. They are often associated with intracranial hemorrhages and/or pneumonia. In this study they were present alone in 25 % of autopsies but when all the infant who had associated findings are included the incidence of hyaline membranes was 40 %. If Idiopathic Respiratory Distress is used as a clinical diagnosis it is not possible to conclude on the basis of autopsy findings whether the clinical diagnosis was correct especially in cases where intracranial hemorrhage or pneumonia are present simultaneously. The evaluation of the significance of intracranial hemorrhage and infection as causes of death and as the principal clinical diseases of premature infants varies with the pathologist. In spite of the difficulties of clinical diagnosis and variations in the evaluation of autopsy findings it is worth while to try to make morphologic clinical diagnoses of the diseases of premature infants.

V. Rasmus. Research on Cystic Fibrosis of the Pancreas in the U.S.A.

In the past ten years, research interest in problems of C.F. has been much increased. This is especially true in the United States where this disease is a fairly common disorder. A general awareness has been created

by activities of the National Cystic Fibrosis Research Foundation. This Foundation has built up a nationwide network of Care, Research, and Teaching Centers in hospitals affiliated with medical schools. Numerous research projects, both clinical and basic are conducted in these centers. Among the principal lines followed only a few might be mentioned: 1) A search for the basic defect in C.F. by biochemical, histochemical and immunochemical methods. 2) Studies aimed at improving diagnostic methods. 3) Clinical investigations concerned with therapy. 4) Research on physiological mechanisms, such as the role played by aldosterone and other adrenal steroids in the regulation of sweat and other secretions. 5) Genetic studies on inheritance and incidence of C.F.

The observation, that duodenal contents and other secretions of patients with C.F. contain a mucoprotein of abnormal chemical structure which is easily denatured and rendered insoluble has been held as a clue to the pathogenesis of the symptoms in this disorder. The characteristic change in the ratio of fucose to sialic acid in mucous secretions of C.F. patients is established in several laboratories. Whether this change reflects a quantitative rather than a qualitative alteration of the normal secretory process is under discussion.

Comprehensive immunologic studies have been performed to locate an abnormal specific constituent in tissue extracts and body fluids of fibrocystic patients by using diffusion in agar or immunofluorescent techniques. The finding that a mucosubstance extracted from stool of C.F. patients might represent a unique compound for this disease is not confirmed as yet and serious doubts have been presented as to the specificity of this substance. A possible quantitative difference has been found between certain urinary or submaxillary proteins of fibrocystic patients and normal children. Of interest is Dr. William Blanc's demonstration that the sera of fibrocystic patients might contain autoantibodies to concretions in the acini and ducts of the pancreas. Autoantibodies have been found also in bronchial mucus by using the gel diffusion technique of Ouchterlony. Whether these antibodies play a role in the pathogenesis of the disease or are a normal response to tissue destruction is uncertain. One of the most recent projects is to investigate the role of the peculiar mucoid Pseudomonas aeruginosa strains often recovered from sputum cultures of patients with C.F. It has been suggested that presence of a metabolic factor in secretions of fibrocystics might favor the growth of these bacteria and induce the mucoid degeneration. Whether or not these strains are unique to patients with C.F. is not known as yet.

As for improvements in diagnostic methods a biopsy technique has been developed by Dr. W. J. Warwick and co-workers at the University of Minnesota. Lip biopsies of the labial salivary gland from patients with C.F. show alterations of glands that are not found in the glands from normal children. The abnormalities observed result from ductal obstruction by inspissated mucus.

Meeting December 5, 1964

C. Cedercreutz. Hypnosis

Various phenomenons of hypnosis were demonstrated. A brief outline of how hypnosis should be used as a symptomatic treatment was given. Hypnoanalytical therapy was discussed. The value of hypnosis as a treatment of enuresis nocturna and other pediatric conditions was illustrated by case reports.

Meeting February 20, 1965

G. Joppich (Göttingen): Problems of Vaccination against Small Pox

J. Kantero, Helsinki.

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From the Children's Hospital, University of Helsinki, Finland

Disaccharidases and Histology of Duodenal Mucosa in Congenital Lactose Malabsorption

by KARI LAUNIALA, PEKKA KUITUNEN and J. K. VISAKORPI

Disaccharide malabsorptions, i.e. intolerances, constitute a well-defined group of diseases. The modern theories on the intestinal splitting of disaccharides, developed mainly by Dahlqvist [7], provide a good physiological basis for the understanding of these diseases.

The diagnosis of disaccharide malabsorption is generally based upon peroral loading tests, and chemical examination of the stools and urine. However the recent development of peroral intestinal biopsy technique renders it possible to make direct measurement of intestinal disaccharidase activities. Direct enzyme assay has been applied, especially as regards adults suffering from so-called acquired lactose malabsorption [1 6, 9 11 L, 13 18 20], and in children and adults suffering from coeliac disease with secondary disaccharide malabsorption [10 21 _3 *7]. In congenital forms of disaccharide malabsorption, enzyme determinations have been made in no more than few cases of sucrose-isomaltose malabsorption [_, 4 16], and in three cases of lactose malabsorption [_ 10 *0]. These studies hav confirmed that disaccharide malabsorptions are caused by congenital or acquired enzyme defects in the intestinal mucosa.

This report is concerned with disaccharidase activities and the histology of duodenal mucosa in two infants suffering from congenital lactose malabsorption.

Case Reports

Patient M. J. male, the youngest child in a family with three children. The family history does not reveal any diarrhoeal diseases. The baby was born at term, weighing 3170 g. Feeding was started with breast milk, but after the first meal watery diarrhoea began.

At the age of 20 days, weighing 2800 g, he was admitted to the Central Hospital of Oulu. The diarrhoea continued until at the age of 39 days breast milk feeding was changed to lactose free diet (Nutramigen[1]), after which the diarrhoea stopped immediately. At that time the baby weight was 2700 g. At the age of months, the baby was transferred to the Children's Hospital, University of Helsinki, for further studies. The patient did well, and weighed 3820 g after 21 days on lactose-free diet. A typical lactose malabsorption was confirmed. Detailed laboratory data are presented in Tables 1 and _. After peroral lactose and cellobiose loads, the patient had diarrhoea. Duodenal biopsy was performed at the age of 8 days (after 39 days on a lactose-free diet). A follow-up study at the age of 10 months revealed that the baby was progressing satisfactorily although lactose malabsorption was still demonstrable.

TABLE 1. *Data of absorption tests and X ray examination.*

	Patient M. J	Patient H. T
3-day fat balance		
Absorption index, %	87	94
Fat excretion, g/day	3.2	0.6
5-hour xylose excretion, %	12.4, 22*	27
Barium meal	Normal	Slight floc culation in ileum

* Control 14 days later

Patient H. T., female, the younger of two siblings. The older sibling suffered from some kind of diarrhoea disease during infancy but tolerated milk well. The patient was born at term and weighed 3600 g. She was breast fed, but suffered from diarrhoea, which apparently began after the first meal. She gained weight poorly. The baby was admitted initially to the Central Hospital in Oulu at an age of 22 days, and 7 days later weighing 2850 g to the Children's Hospital, University of Helsinki. A typical lactose malabsorption was found (Tables 1 and 2) and the baby was transferred to a lactose-free diet, on which she showed a satisfactory gain. The patient reacted to lactose and cellobiose peroral loads with marked diarrhoea, but tolerated other sugars satisfactorily. A biopsy was performed at the age of 4½ months (after she had been on a lactose-free diet for 3½ months), the biopsy was repeated 10 days later.

Methods

The duodenal biopsies were effected with a multipurpose suction biopsy tube (Brandborg–Rubin tube) from the distal duodenum. Two mucosal samples were taken. One of those samples was fixed in formalin, and PAS, van Gieson and hematoxylin–eosin stainings were employed. The other sample was immediately frozen at −20°C until the homogenization and enzyme assay could be carried out, as described previously [8, 16]. In patient M. J. two samples were obtained at the same biopsy. In patient H. T. one mucosal

TABLE 2. *Findings after peroral sugarloads*

	Patient M. J	Patient H. T
Glucose–galactose load		
Increase of blood-sugar content, mg/100 ml	38	49
Highest glucose + galactose content in stools, mg/g d.w	6	3
Highest glucose + galactose content in urine, mg/100 ml	5	20
Lactose load		
Increase of blood-sugar content, mg/100 ml	0	4
Highest lactose content in stools, mg/g d.w	409	796
Highest lactose content in urine, mg/100 ml	60	20
Cellobiose load		
Increase of blood-sugar content, mg/100 ml	11	0
Highest cellobiose content in stools, mg/g d.w	401	619
Highest cellobiose content in urine, mg/100 ml	15	8
Sucrose load		
Increase of blood-sugar content, mg/100 ml	64	62
Highest sucrose content in stools, mg/g d.w	0	0
Highest sucrose content in urine, mg/100 ml	8	5

Loads were performed after 8 hours fasting with dosis 2 g/kg disaccharide or 1 + 1 g/kg monosaccharide.

Table 3 *Disaccharidase activities of duodenal mucosa (units/g protein).*

	Patient M. J	Patient H. T	Controls[b] Range (No. of patients)	Normal adults (Auricchio *et al.* [3], Biopsy Material). Range (= 10)
Maltase	296	231	135–1215 (12)	310–1120
Sucrase	103	76	33– 306 (12)	70– 325
Isomaltase	106	102	46– 254 (6)	63– 264
Trehalase	35	12	12– 120 (7)	—
Lactase	<1	<1	16– 120 (12)	29– 58
Cellobiase	<1	<1	1.4– 27 (10)	9– 1

One unit of disaccharidase activity causes hydrolysis of one micromol disaccharide in 1 min at 37°C and at optimum pH

Controls are infant and children suffering from chronic diarrhoea and malabsorption syndrome.

piece was obtained initially and this was used for enzyme assay A second biopsy was performed 10 days later and the mucosal sample thus acquired was subjected t histological staining

Other laboratory determinations were made by routine methods. Peroral sugar loads were performed with various sugars, 2 g per kg following an 8 hour fast, and blood sugar was estimated with o-toluidine method. After loading, urine and stools were collected. The chemical analysis of stools and urine was effected as has been described elsewhere [17].

Results

A. *The disaccharidase acti ities of duodenal mucosa* (Table 3). Th results of the disaccharidase assays demonstrate that lactase and cellobiase ctivities were practically absent in both patients studied, whereas other disaccharidase activities appeared to be normal when a comparison was made with the control values of the present authors, and with the normal values presented by Auricchio *et al.* [3]. Large variations in individual disaccharidase activities exist as is observable in the control material. Nevertheless, the ratios between the different disaccharidases are rather constant In the patients reported on here the maltase, isomaltase, sucrase and trehalase activities were all at a mean normal level, which makes the very low lactase and cellobiase activities even more significant.

B. *The histology of duodenal mucosa.* In both patients the biopsy samples were taken from the distal duodenum. In the sample taken from patient M. J., normal villi were seen. Five villi of 6 completely visible were longer than 300 μ; one villus was about 200 μ. The columnar epithelium was intact. The striated border and the basement membrane were distinct the nuclei were regular and no increased cellular infiltration was discoverable in the lamina propria (Fig 1).

In the sample taken from patient H. T villi seem to be slightly shorter and broader than normal. The villous length was bout 170–276 μ. The columnar epithelium of the villi was normal. The striated border and the basement membrane were clear the nuclei were regular and no increased cellular infiltration was observable in the lamina propria (Fig 2).

Thus the duodenal mucosa of patient



Fig. 1. (*a*) Histologic section of duodenum from patient M. J. showing normal mucosa (× 120). (*b*) *Note the intact striated border and the intact basement membrane* (× 240). *PAS-van Gieson stain.*

M. J. was completely normal, although in the sample taken from patient H. T. slight partial villous atrophy was apparent.

Discussion

The histories of both patients presented in this paper revealed diarrhoea, which began after the first breast milk feeding. In both cases this finding suggested lactose malabsorption from the beginning. Both the clinical course after lactose elimination and the oral loadings with various sugars clearly confirmed this diagnosis. Other tests for the measurement of intestinal absorption function gave normal results, except as regards the xylose excretion test in patient M. J. However a



Fig. 2. (*a*) Histologic section of duodenum from patient H. T. showing slight partial villous atrophy (× 120). (*b*) Note the striated border and the basement membrane are fairly well defined. Slight mononuclear cell infiltration can be seen in the lamina propria (× 240). PAS-van Gieson stain.

control test made two weeks later gave a normal result. These absorption tests excluded more generalized malabsorption. Thus the diagnostic criteria of congenital lactose malabsorption, as presen ted by Prader *et al.* [22], are met in these cases.

Enzyme determinations revealed almost absent lactase and cellobiase activities in both cases, a finding which was well in accord with the clinical findings: distinct malabsorption of lactose and cellobiose At the same time, the defect in lactase and cellobiase is in close accord with the experimental studies made by Dahlqvist [7] and Semenza *et al.* [23 24]. They demonstrated by heat inactivation and by column chromatography that lactase and cellobiase are identical enzymes. In addition, Semenza *et al.* [23, 24] demon strated that lactase–cellobiase activity can be separated into two fractions with column chromatography In the first, major fraction the ratio between lactase cellobiase was five, and in the second, minor fraction this ratio was two

Auricchio *et al* [ ] studied a 5-year-old boy with congenital lactose malabsorption and found diminished lactase activity (5 7 U/g of protein) and diminished cellobiase activity (_ U/g of protein). Since in their case the ratio between the remaining lactase and cellobiase activities was _6, they suggested that their patient presented a deficiency of the first major lactase fraction, and that the low lactase activity found was attributable to the second fraction In both of the cases pre sented here lactase and cellobiase activities were almost absent, suggesting a lack of both lactase–cellobiase fraction

As regards ther patients with congenital lactose malabsorption where enzyme assay has been performed, the patient of Cozzetto [5] had a comparatively high lactase activity (40 U/g of protein) although the ratio between lactase and sucrase was clearly pathological. No numerical data have been published in respect of the patient reported by Sobel *et al.* [26]. Enzyme assays in acquired lactose malabsorption (adults) have been reported by many authors [1 6, 9 11 L 13 18 20] and similar low activities have been found. The lactase activity in secondary disaccharide malabsorption associated with coeliac disease or sprue is diminished, but not to such an extent as in primary lac tose malabsorption [19, 21 _5]. On the basis of all these studies, it can be tated that clinical lactose (and cellobiose) malabsorption is combined with more or less complete enzyme deficiency in intestinal mucosa.

There is little information on the intesti nal mucosal histology in congenital di saccharide malabsorption. A priori, there may be assumed to be a normal small bowel mucosal histology as generalized malabsorption is not usually found in patients with primary disaccharide malabsorption. Cozzetto [5] found a normal mucosa in his patient with congenital lactose malabsorption with cystic fibrosis Lamy *et al.* [15] demonstrated a subtotal villous atrophy in a patient with congenital sucrose–isomaltose malabsorption They also found that these mucosal changes became completely normal after _ months treatment with elimination diet. Kuitunen [14] found normal intestinal mucosa in a similar patient with sucrose-isomaltose malabsorption, altho gh this patient had been under treatment for

several months already In acquired lactose malabsorption, normal histology of the small bowel has been found [6, 11 12, 13 18 20]

One of our patients demonstrated a normal histology of the duodenum and the other one slight atrophic changes Since, both patients had been on a lactose-free diet, no conclusions can be drawn regarding the histological picture at the diarrhoeal stage. A normal absorption test, faecal fat and xylose excretion tests provide evidence of rather normal mucosa also in an active state of the disease Slight changes seen in the second case may be secondary results of long lasting diarrhoea

Summary

A study has been made of the disacchari dase activities and histology of duodenal mucosa in two infants with typical con genital lactose malabsorption. The mucosal samples were taken by means of peroral dnal biopsy At the time of biopsy patients were 2½ and 4½ months old and they had been on lactose free diet 39 days and 3½ months respectively

Both patients demonstrated a typical clinical picture of lactose and cellobiose malabsorption. In the mucosal samples, lactase and cellobiase activities were almost absent in both patients, whereas other disaccharidase activities were within the normal range. The histological picture of the mucosa was normal in one patient, and slight, partial villous atrophy was seen in the other

This study confirms that the cause of congenital lactose malabsorbtion is an enzyme defect of the intestinal mucosa which histologically may appear normal

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References

1 AURICCHIO, S., RUBINO, A., LANDOLT, M., SEMENZA, G and PRADER, A.: Isolated in testinal lactase deficiency in the adult. *Lancet, II* 324, 1963.

2 AURICCHIO, S., RUBINO A., PRADER, A. REY J JOS, J FREZAL, J and DAVIDSON M. Intestinal glycosidase activities in congenital malabsorption of disaccharides. *J Pediat, 66* 555, 1965.

3. AURICCHIO, S., RUBINO, A., TOSI, R. SEMENZA, G LANDOLT, M. KISTLER, H J and PRADER, A.: Disaccharidase activities in human intestinal mucosa. *Enzymol biol clin, 3.* 193, 1963.

4. BURGESS, E. A., LEVIN B., MAHALANABIS, D and TONGE, R. E.: Hereditary sucrose intolerance: levels of sucrase activity in jejunal mucosa. *Arch Dis Child, 39* 431, 1964

5. COZZETTO, F J Intestinal lactase deficiency in patient with cystic fibrosis. *Pediatrics, 32:* 228, 1963.

6. CU TRECASAS, P., LOCKWOOD D H. and CALDWELL, J R Lactase deficiency in the adult. A common occurrence. *Lancet, I* 14, 1965.

7 DAHLQVIST A. Specificity of the human intestinal disaccharidases and implications for hereditary disaccharide intolerance. *J Clin Invest, 41* 463, 1962.

8. — Method for assay of intestinal disaccharidases. *Anal Biochem, 7* 18, 1964.

9 DAHLQVIST A., HAMMOND J B CRANE, R. K., DUNPHY J V and LITTMAN A Intestinal lactase deficiency and lactose intolerance in adults. *Gastroenterology 45* 488, 1963.

10. DAVIDSON, M. SOBEL, H. H. KUGLER, M M and PRADER, A. Intestinal lactase deficiency of presumed congenital origin in two older children. *Gastroenterology 46* 737 1964

11 DUNPHY J V LITTMAN A., HAMMOND J

B., Forstner, G. Dahlqvist, A. and Crane, R. K.: A syndrome of lactose intolerance and lactase deficit in adults. *Gastroenterology, 46* 729 1964.
12. Haemmerli, U. P., Kistler, H. J., Ammann, R., Auricchio, S. and Prader, A.: Lactasemangel der Dunndarmmucosa als Ursache gewisser Formen erworbener Milchintoleranz beim Erwachsenen. *Helvet Med Acta 29* 693, 1962.
13. Haemmerli, U. P., Kistler, H., Ammann, R., Marthaler, T. Semenza, G., Auricchio, S. and Prader, A.: Acquired milk intolerance in the adult caused by lactose malabsorption due to a selective deficiency of intestinal lactase activity. *Amer J Med 38* 7 1965.
14. Kuitunen P.: Histology in malabsorption syndrome in infancy. To be published.
15. Lamy M., Nezelof C. Jos, J. Frézal, J and Rey J. La biopsie de la muqueuse intestinale chez l'enfant. Premiers résultats d'une étude des syndromes de malabsorption. *Presse Méd, 71*: 1267 1963.
16. Launiala, K., Perheentupa, J., Visakorpi, J and Hallman N. Disaccharidases of intestinal mucosa in a patient with sucrose intolerance. *Pediatrics 34* 615, 1964.
17. Launiala, K.: To be published.
18. McMichael, H. B., Webb, J and Dawson A. M.: Lactase deficiency in adults. A cause of "functional" diarrhoea. *Lancet, I* 717 1965.
19. Nordio, S., Lamedica, G. M., Vignolo, L. and Berio, A.: Six cases of lactose intolerance. Lactose intolerance and coeliac disease. Disaccharidases activity in the intestinal mucosa ascertained with the peroral biopsy. *Ann Paediat* (Basel), *204* 3, 1965.
20. Peternel, W. W. Lactose tolerance in relation to intestinal lactase activity. *Gastroenterology 48*, 299 1965.
21. Plotkin, G. R. and Isselbacher, K. J. Secondary disaccharidase deficiency in adult celiac disease (nontropical sprue) and other malabsorption states. *New Eng J Med, 271* 1033, 1964.
22. Prader, A., Semenza, G. and Auricchio S.: Intestinale Absorption und Malabsorption der Disaccharide. *Schweiz Med Wschr 93* 1272, 1963.
23. Semenza, G. and Auricchio, S.: Chromatographic separation of human intestinal disaccharidases. *Biochim Biophys Acta 65* 172, 1962.
24. Semenza, G. Auricchio, S. and Rubino, A.: Multiplicity of human intestinal disaccharidases. I. Chromatografic separation of maltases and of two lactases. *Biochim Biophys Acta, 96*, 487 1965.
25. Shmerling, D. H., Auricchio, S. Rubino, A., Hadorn, B. and Prader, A.: Der sekundäre Mangel an intestinaler Disaccharidaseaktivität bei der Cöliakie. Quantitative Bestimmung der Enzymaktivität und klinische Beurteilung. *Helvet Paediat Acta 19* 507 1964.
26. Sobel, E. H. Davidson M., Kugler, M. M., Zuppinger, K. A., Yu Feng Hsu L. and Prader, A.: Growth retardation associated with intestinal lactase deficiency *J Pediat, 62*, 731 1963.
27. Weser, E. and Sleisenger, M. H.: Lactosuria and lactose deficiency in adult celiac disease. *Gastroenterology 48* 571, 1965.

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Vaccinia Gangrenosa with Normal Humoral Antibodies

A Case Possibly Due to Deficient Cellular Immunity Treated with N Methylisatin β-Thiosemicarbazone (Compound 33T57 Marboran)

by O HANSSON S G O JOHANSSON and B. VAHLQUIST

Vaccinia gangrenosa is a feared complication of smallpox vaccination. Until recent times it has been associated with high mortality Patients with vaccinia gangrenosa often have specific immunological deficiencies, which may however vary in character

In the following a case of vaccinia gangrenosa will be described, where the essential deficiency appears to lie in the cellular immunity The presentation will [in]clude a report on therapy with the new [a]ntivariola agent N methylisatin β-thiosemicarbazone (Compound 33T57 Marboran Burroughs Wellcome & Co. London) which in this case appears to have had a life-saving effect.

Case History

G H a girl, born Jan 6, 1962. Only child of healthy parents. No relatives with known antibody deficiency disease. Normal delivery Birth weight 3000 g. BCG vaccination in the newborn period. Tuberculin test at 2 months of age was negative. Immunized with combined vaccine (Triple antigen) three times between 3 and 6 months of age without any abnormal reaction. Earlier history noncontributory

On Sept. 19 1962, she was vaccinated against variola on the lateral side of the left upper arm. Initial course was normal. After two weeks the inoculation sit showed an infected ulcer with secondary pustules. The girl was subfebrile but otherwise in good condition. During the following weeks the ulcer showed no tendency to heal. She was then admitted to the local hospital Oct. 17 1962. On physical examination she appeared to be in good condition and was afebrile. The ulcer on the left upper arm was infected and measured 2 5 cm. Its circumference showed a broad reddened margin with swelling and induration. In addition to several secondary pustules around the ulcer and on the left shoulder she had one pustule on the left thigh and a few on the neck and in the scalp. Some minor lymph nodes were palpable in the anterior cervical and axillary regions. Liver and spleen were not palpable.

Haemoglobin was 11.3 g/100 ml, red cell count 4.3 million, and white cell cou t 6200 (non-segmented neutrophils 7 %, poly morphonuclears 21 %, eosinophils 39 %, basophils 1 %, lymphocytes 18 %, monocytes 14 %) The neutrophils showed toxic granulation. The micro ESR was 52 mm The serum-electrophoresis was normal. Urinalysis was normal

Systemic treatment with penicillin chloromycetin, sulphonamides, "Tanderil (oxfenbutazon) and two injections of ordinary gamma globulin (a total of 18 ml of a 12









Fig. 1. Serial pictures of gangrenous ulcer ( ) At admission, 4 months after vaccination (23.1.63). (b) 2 weeks after surgical excision (18.2.63). (c) 4 weeks after the 2nd course of Compound 33T57 (29.3.63). (d) 3 months after skin utograft (4.7.63).

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suspension) was given in addition to local treatment. The secondary pustules on the left leg, the neck and in the scalp healed, but the necrotic ulcer showed steady slow deterioration. On Jan. 21 1963, four months after vaccination, the girl was transferred t the Department of Pediatrics, Uppsala.

O admission, the patient was in good general condition. Her left upper arm was heavily swollen, but not tender The inoculation site showed a necrotic ulcer measuring 3 5 cm with a depth of cm. The edges were discoloured, swollen and indurated. Around the ulcer and on the left shoulder were several secondary pustules. Apart from slight enlargement of the lymph nodes in the left axilla the physical status was otherwise normal.

Treatment and course. Vaccinia gangrenosa was diagnosed and in accordance with many earlier described cases it was assumed that she lacked neutralizing antibodies against vaccinia irus. Treatment with hyperimmune vaccinial gammaglobulin was therefore initiated in a dose of 10 ml every second day She recei ed a total amount of 410 ml. Broad spectrum penicillin (Ampicillin 2.0 mg 4) was given simultaneously The expected improvement failed to take place however and for a short period of time Salazopyrin (0.5 g 4) was added, but still no effect was observed. Assuming inadequat blood supply to the swollen and indurated tissues as the reason for the therapeutical failure, surgical excision was performed on Feb. 4, 1963. N improvement followed the operation; on the contrary her local as well as her general condition deteriorated considerably

At this phase of the disease it was possible due to the courtesy of the Wellcome Laboratories of Tropical Medicine London (Dr D J Bauer), to commence treatment ith Compound 33T57 (N methylisatin β thiosemicarbazone) on Feb. 10, 1963 As recommended a total amount of 0 ml f 10 suspension was gi en by mouth ov a period of 4½ days. Within 48 hours a pronounced decrease of the peripheral oedema of the left forearm was noted and the edges of the ulcer seemed hyperaemic. A few days later granulation tissue was seen to grow from the upper edge towards the centre of the ulcer

However tw lve day after the Compound 33T57 treatment was finished a secondary vaccinia pustule appeared on the left shoulder on the sit f previously healed pustule. For a few day prior to this the granulation tissue had ppeared t stop growing. Another course of Compound 33T57 was now given in the same dosage. Again a prompt effect was seen, the secondary pustule disappeared within 12 hours and the granulation tissue was reactivated. No side effects of the treatment were observed. A steady improvement was noted from now on. April 4, 1963, a skin autograft was performed, which, however did not take. Three weeks later another attempt was made with complete success (F g 1)

During her entire stay in hospital she was afebrile but for a long period bedridden. When mobilization was started after successful skin graft a considerable retardation of psychomotor development was noted, and an atactic coordination disturbance was aguely suspected, EEG was normal. X-ray films of the left upper arm showed no skeletal changes. Tuberculin test was negati e for 1 mg She was discharged May 27 1963

Laboratory tests. Haemoglobin was 12.6 g/100 ml. Whit cell count was 000 (non segmented neutrophils 1.5 ,, polymorphonuclears 60.5 ,, eosinophils 15.0 ,, basophils 0 lymphocytes 18.3 ,, monocytes 4.5). The eosinophilia which initially reached a maximum of 39 decreased parallel to clinical improvement. The micro-ESR was 16–51 9 mm. Bone-marrow biopsy showed a marked increase of eosinophilic cells in different stages of development and sparse plasma cells. Urin analysis was normal. Vaccinia irus was cultured from the ulcer Bacteriological cultures showed growths of Pyocyaneus, Staphylococcus aureus and β-haemolytical Streptococci.

Serological investigations. Antistaphylolysin 4.5 units/ml and antistreptolysin 500

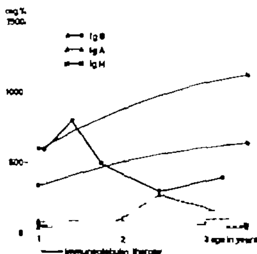


Fig. 2. Immune globulin concentration during and after the course of the disease.

units/ml. Agglutination titre against pertussis antigen was positive in serum dilution 1/100. Tetanus-antitoxin > 1 < 10 IU/ml. Diphtheria-antitoxin 0.7 IU/ml. The 50 % neutralization titre against vaccinia virus was 1:500 (about the same as for hyperimmune vaccinial gamma globulin); there was 93 % neutralisation against 100 plaques-units of vaccinia virus in serum dilution 1/125. After treatment with hyperimmune vaccinial gamma globulin the titre was on the same level. Haemagglutination-inhibition test against vaccinia virus was positive 1/80. Complement fixation test was negative. BCG-induced antibodies were not demonstrable in serum (State Serum Institute, Copenhagen).

The patient belonged to blood group B Rh(+). Isoagglutinins but no irregular antibodies against red blood cells could be demonstrated. The direct antiglobulin test (Coombs) was negative.

Immunochemistry. The presence of immune globulins was determined qualitatively by immunoelectrophoresis according to the method of Scheidegger with minor modifications [23], and quantitatively by passive haemagglutination [20].

The IgG, IgA and IgM globulin quantities obtained by immunoelectrophoresis agreed well with the results obtained by the haemagglutination inhibition technique. Immune globulin concentrations on different occasions during and after the course of the disease are shown in Fig. 2. The corresponding values given for healthy children were obtained from other publications [16, 20, 24]. Serum from five children hospitalized for conditions other than infections and blood diseases was examined by the haemagglutination inhibition method, and the values obtained conformed with the normal values stated.

Interferon. (Kindly performed by Drs. L. Philipson and S. Harmodsson.) Interferon production by leucocytes from the patient was assayed according to the procedure of Gresser [15] with Parainfluenza virus type 1 (Sendai) as challenge virus. For control purposes leucocytes from a control child and an adult were tested. Two samples of about 10^6 leucocytes were tested 6 weeks apart. The first sample from the patient produced interferon to a reciprocal titre of < 4 of the medium compared to 64 of an adult control. In the second sample the reciprocal interferon titre was 8 compared to 32 of a control child and 24 of an adult control. The leucocytes from the same adult were tested on the two occasions.

Readmission. In Sept., 1963 when the girl was readmitted for further plastic surgery she showed definite signs of an ataxic diplegia but was otherwise in good condition. EEG and EMG were normal. Routine laboratory tests were normal.

An attempt at homologous skin transplantation was made together with the plastic surgery. However, the graft was rejected three weeks later.

Intracutaneous tests with vaccinia antigen [28] were negative in 1/1000 to non diluted solutions.

Discussion

Immunology

The smallpox vaccination in the present case was performed at the customary age and with a vaccine which is carefully

standardized as regards its antigenic strength [13]. This alone justifies the assumption that the divergent course was due to a constitutional abnormality in the child. The results of the immunochemical and serological investigations, examination of the blood picture and tissue immunity tests are summarized in the following.

Immunochemistry. During the period which the patient spent in the Pediatric Clinic, University Hospital, Uppsala, the concentrations of IgG, IgA and IgM globulin in serum were essentially normal. The relatively high IgG globulin concentration which was observed in the serum from May 1963 is probably explained by the large doses of immune globulin against vaccinia which the patient had received. A total of approximately 50 g IgG globulin was given within a period of 11 weeks.

During the post-disease period the IgG globulin concentration appears to have become stable at a level somewhat lower than that stated as normal for her age. This is remarkable in view of the infection which she has undergone. The IgM globulin concentration is essentially normal.

Also remarkable are the high concentrations of IgA globulin which were shown in the serum from June 1964 and March 1965. Increases of IgG and IgM globulin concentrations may be seen in the period following infectious conditions but usually then occur shortly after recovery. In the present case the increase is late. The IgA globulin concentration was not raised 4 months after the disease while after 12 months it was about 4 times higher than the normal value, and after 20 months within normal limits. No test was made between 4 and 12 months after the disease period.

High IgA globulin concentrations such as these can represent extreme normal values for the age. It does not seem improbable, however, that in this case the increase could be due to delayed immunological response to the vaccinia infection.

Serology. Isoagglutinins were demonstrated in normal titres. Antistaphylolysin and antistreptolysin were shown to be present.

The antibody response to the different vaccinations is of special interest. As seen in the case history data the antibody response to triple vaccination was normal. With regard to the antibody response to vaccinia the child showed in the investigation carried out 4 months after the vaccination and before commencement of treatment with hyperimmune vaccinial gamma globulin a titre of 1/80 for haemagglutination/inhibition and 1/1000 for 50 % neutralization. These figures lie above the mean values for a normal vaccination response. No BCG induced antibodies were demonstrated. The BCG reaction is discussed below under the heading of tissue immunity.

Leucocyte picture. Most worthy of note was the pronounced and persistent eosinophilia, with a maximum value of 39 % (=2418 cells/mm³). Parallel with the general clinical improvement the eosinophilia gradually receded and at the latest follow up examination one year after recovery the differential count was normal. The eosinophilia was thus not constitutional in this case but must have been a reaction to the disease. The neutrophil granulocytes showed extreme values of between 9100 and 1300 and the lympho-

This has been observed in isolated cases, however

The future prognosis in our case, and especially the possible reaction to further vaccinations, is very difficult to forecast. As regards variola vaccination it is clear that revaccination should not be performed. Other viral vaccinations should *probably be avoided unless absolutely* necessary Whether the girl runs the risk of an abnormal course in ordinary viral infections is theoretically difficult to determine—probably not, however

Therapy

In those cases where the main cause of the vaccinia gangrenosa has been a deficiency of specific antibodies, either isolated or as a general deficiency in antibody production, the obvious and most important form of therapy has been the administration of immune gamma globulin in large doses. About 30 cases have been described, 23 of them by Kempe alone [22], where this therapy has been tried, in many cases with a decided improvement in the course of the disease The reasons for failure of this treatment in certain cases may have varied. the disease has progressed too far the antibodies administered have not reached a sufficiently high concentration (either generally or in the gangrenous ulcer zone) and/or a primary deficiency in tissue immunity has been present in addition to the humoral deficiency

In our case the spontaneous antibody titres were high, and we therefore have no reason to believe that there was any deficiency in humoral immunity No effect was observed from treatment with immune gamma globulin, even though it was given in very large doses, a total of 410 ml.

In our case no trial was made with local injection of lymphocyte concentrate from healthy donors into the tissues surrounding the ulcer nor of the administration of human embryonal tissue [35] On the other hand the new chemotherapeutic prepared by Burroughs Wellcome & Co London, and which has shown a remarkable effect against variola and vaccinia virus [3, 5 10 11 31 33] was tested.

Since in February 1963 the disease inexorably and despite all therapeutic attempts continued to progress, contact was made at this time with Dr Bauer who arranged a delivery of Compound 33T57 As mentioned in the case report the effect was dramatic, and as early as within 48 hours the vaccinia pustules had dried up and a tendency to incipient granulation in the ulcer margins could be discerned. Twelve days later after a further 5-day course had been given, a definite and final improvement had taken place.

It is not permissible to generalize from isolated observations. For us, however who at close quarters followed the dramatic change within the course of two days, after months of unsuccessful attempts at therapy it is very difficult not to believe in a true causative factor Of the four cases of vaccinia gangrenosa treated with Compound 33T57 who were reported at the time of our preliminary communication [17], two recovered (both were adults with acute leukemia and *giant follicular* lymphoma respectively [10 11]) while two infants died despite the therapy [9 35] If the pathogenesis is analysed in these latter cases, as far as possible from the data presented, it is obvious that in

one of them [35] there was a thymic aplasia with agammaglobulinaemia. Such cases are well known for their poor prognosis and usually (even without the additional influence of vaccinia) fatal outcome *during the first year of life.*

Compound 33T57 is a new potent chemotherapeutic drug. The question must thus arise, especially with regard to individuals of an early age, as to whether it may have any toxic side-effects. In our case the last follow up at the age of 2½ years revealed signs of CNS damage of the atactic diplegia type. There is reason to believe that the girl was completely healthy up to the age of 8 months, with reservation for possible discrete functional disturbances, which must always be made for children of this age. There was no information of any perinatal damage in the patient nor of any early manifested CNS disease among the relatives. It is therefore reasonable to assume that the CNS affection occurred during the period between the onset of vaccinia gangrenosa at 8 months and the time when the CNS symptoms were first suspected, i.e. at 15 months. This period includes many months of severe disease and treatment of various kinds. The general condition was affected to a remarkably small extent even when the local disease was pronounced, there was hardly any febrile reaction and signs of acute *encephalitis were never observed.* Nevertheless an undiagnosed subacute vaccinia *encephalitis may very conceivably* explain the neurological damage. Even the local gangrenous disease process may have affected the central nervous system, the pronounced eosinophilia, for instance, indicates general involvement.

For 3–4 months the therapy consisted of a large selection of preparations. Most of the drugs used can probably be dismissed as atoxic, but a few remain, viz., "Salazopyrine", "Tanderil" and Compound 33T57. CNS damage of the type *seen in the present case has not, however,* been reported previously in connection with these preparations. As regards Compound 33T57, no symptoms have been observed in animal experiments and in voluntary experiments on healthy adults [4].

Is it possible to avoid vaccinia gangrenosa by eliminating special risk cases *from smallpox vaccination?* Obviously as far as cases with established agammaglobulinaemia are concerned, even if the vaccination may be free of complications, the possibility of their occurring must be taken into account. On the whole it is probably wise not to give smallpox vaccinations to children who have shown a tendency to severe recurrent infections or who have had disease patterns of the autoimmune type. A case such as ours could never, however, be *eliminated in this way.* It must be assumed therefore that even when smallpox vaccination is carried out under the apparently most favourable conditions, vaccinia gangrenosa can occur in rare cases. It is then important to have access both to immune gamma globulin and chemotherapy of the type represented by Compound 33T57.

Summary

A case of vaccinia gangrenosa with normal antibodies and deficient cellular immunity against vaccinia-antigen is presented. Administration of hyperimmune vaccinial gamma globulin failed to stop the progression of the disease. Treatment with Compound 33T57 was successful.

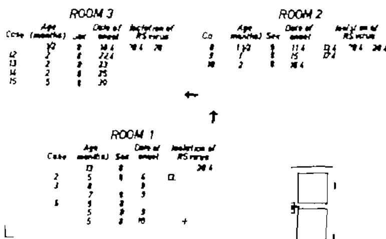


Fig. 1 Age, sex, date of onset of illness and virus findings in 15 children with RS virus infection.

holm. The hospitalized children were also examined by us (G. S. and G. de H.)

An aetiological study was started on April 12, 1964 as shown in Fig. 1

Virological studies

Isolations from pharynx and nasopharynx were made at the bedside, the swab being immediately soaked in the medium of a tube culture of HeLa cells. Cultures of HeLa cells, in tubes and flasks, were prepared as described elsewhere [25] All cultures were incubated at 35°C.

The roller tube cultures used for virus isolation were maintained on bovine amniotic fluid with the addition of 0.5 per cent bactotryptose, 100 units of penicillin and streptomycin, 50 units of neomycin and 25 units of mycostatin. Typical cytopathic changes appeared after 4–10 days. In subpassages 5–10 per cent guinea pig serum (heated to 56°C for 30 min) was added to the medium but no additions of mycostatin were made. Isolates were typed by complement fixation and in four cases also by neutralization tests against sera from guinea pigs immunized with the prototype Long strain. In eight cases the serum used in the CF tests was from animals inoculated only once intranasally; it was adsorbed with noninfected tissue culture material before being employed. In the remaining five cases the serum used in the CF test was from animals inoculated intraperitoneally three times. The serum was subsequently not absorbed. Serum from hyperimmunized animals was used in neutralization tests. Antigens for CF typing were prepared in flasks or tubes with HeLa cells maintained with bovine amniotic fluid containing bactotryptose antibiotics and 5 per cent inactivated guinea pig serum. When cell destruction was complete after 8–14 days, the contents of the flasks or tubes was frozen and thawed three times. It was concentrated (flasks only) by forced dialysis against polyethylene glycol (Carbowax 20 M, Union Carbide Chemical Co.) [17] before being titrated against 8 CF antibody units.

RS antigen for serological tests was prepared similarly although the Long strain was employed, the concentration procedure was omitted and the antigen was heat inactivated (56°C 30 min) Sera were tested against two as well as four optimal units of Long antigen; no significant difference in results was obtained. Sera were in addition

screen diluted ¼ for CF antibodies against influenza A and B (group), parainfluenza 1 2, and 3, mumps, adenovirus (group), herpes simplex and measles CF antigens. Positive sera were then titrated starting with dilution of ¼ In all CF tests the method of Fulton and Dumbell [9] as modified by Svedmyr *et al.* [23] was used.

Bacterial study

Nasopharyngeal and throat swabs for bacterial culture were taken from all infants and six members of the staff simultaneously with the first swabs for virus isolation. Pneumococci, streptococci, staphylococcus aureus and Hemophilus influenzae were especially looked for

Results

Virological findings

As seen in Fig. 1 and Table 1 RS virus was obtained from 13 out of 15 infants. There was no evident difference between the occurrence of virus recovery from throat swabs compared with nasopharyngeal swabs. RS virus was isolated during a period from days before (case 15) to 9 days after onset of illness (case 8). In the two infants (cases 1 and 4) from whom RS virus could not be recovered, CF antibody titre against RS virus rose significantly (>4-fold) (Table 1). In addition CF antibody titre against RS virus showed at least a 4-fold rise in five other cases. In four of these children, however the highest titre did not xceed 4 One infant (case 11) and perhaps two others (cases 12 and 14) had antibodies against RS virus before their illness, possibly of maternal origin.

In case 1 the attempt at virus isolation was made as late as 16 days after the onset of illness. However this was not until 13 days after the child was admitted to hospital due to pneumonia. Adenovirus type 3 was then recovered from the nasopharyngeal specimen but no RS virus. A similar specimen taken one week later

TABLE 1 *Virological findings in 15 infants with RS virus infection*

Case No.	Age months	RS virus isolated	Complement fixing antibodies Serum 1	Serum 2	Serum 3
1	13	Neg.	< 2 (9)	8 (31)	
2	6	+	< 2 (7)	2 (21)	
3	8		2 (4)	16 (26)	
4	7	Neg	< 2 (4)	16 (28)	
5	3	+	< 2 (4)	< 2 (18)	
6	3		2 (4)	4 (15)	
7	5		< 2 (3)	4 (17)	
8	1½	(9)	< 2 (2)	4 (16)	
9	1		< 2 (2)[a]	< 2 (12)	
10	2		2 (3)[a]	< 2 (11)	< 2 (19)
11	1½	+	8 (4)[a]	4 (9)	16 (17)
12	2		2 (9)[a]	2 (6)	4 (23)
13	2		2 (10)[a]	< 2 (4)	< 2 (24)
14	2		2 (12)[a]	< 2 (2)	< 2 (18)
15	5	(2)[a]	< 2 (17)[a]	< 2 (11)	4 (19)

( ) days after onset of illness.
( ) days before onset of illness.

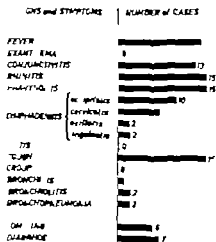


Fig. 2. Signs and symptoms in 15 children (1–13 months of age) with RS virus infection.

contained adenovirus type 5. There was no significant rise in CF antibody titre against adenovirus in this case or in any of the others. Nor could any significant rise be demonstrated in CF antibody titre against the other antigens used except RS virus, as mentioned above.

Neutralizing antibodies against RS virus were only determined in two infants (cases 4 and 5). A significant rise was demonstrated in both cases.

No virus strains were isolated from the staff members studied. No rising CF antibody titres against RS virus occurred in this group, but in all sera the titre was > 4.

Bacteriological findings

Staphylococcus aureus was the only potentially pathogenic organism isolated. It was recovered from four of the youngest infants.

Epidemiological observations

As mentioned above all infants as well as the members of the staff, were healthy during the week prior to April 4, 1964. The first infant who fell ill was a boy 13 months old (case 1). He had been treated for strophulus and anaemia in the Children's Hospital Samariten during the last 3 weeks of March and the first 2 days of April 1964. As far as we know, none of the other children or the staff members in the hospital ward, where this boy stayed, had been ill with acute respiratory infection during the week before April 3, 1964. However, all parents were permitted to visit their children freely providing an opportunity of virus transmission. The boy returned from the hospital on April 3. He had no clinical signs of illness, when he arrived at the children's home. However, on the following day (April 4) he fell ill with fever, nasal discharge and cough. After 3 days with high temperature, rales were found over the chest. He was sent back to the hospital. X rays of the chest showed bronchopneumonia. Paired sera from this child (case 1) showed a significant rise in CF antibody titre against RS virus (Table 1).

As seen from Fig. 1 the other infants became ill between April 6 and April 30. The incubation period appeared to be from 3 to 5 days. In one infant (case 15) RS virus was isolated 2 days before the onset of illness. It is therefore not unlikely that the incubation period in case 2 also (Fig. 1) was 3 days.

Clinical findings

All infants developed signs of acute respiratory illness (Fig.), with rhinitis, pharyngitis, and a cough which was slightly hoarse. The conjunctivitis was slight and non purulent. It was remarkable how often occipital lymphadenitis occurred. Wheezes were heard in three infants including one with the diagnosis of bron

chitis (case 9) and two with bronchiolitis (cases 10 and 14). The last mentioned infants were critically ill and had to be treated in oxygen tents. Chest X ray was normal in these three cases. Two infants (cases 1 and 8) had fine rales over the chest and nodular densities were demonstrated in the lungs of both cases by X ray. One of them (case 8) was the only one who had normal temperature throughout the illness. She had a light nasopharyngitis with cough during the first two days. Suddenly on the third day this infant became severely ill with dyspnoea, cyanosis and fine rales over the chest. The child was immediately admitted to hospital. An X ray showed nodular densities in the lungs, as mentioned above.

Table 2 shows the highest temperature and the duration of fever (≥37.4 C).

Seven children had diarrhoea, which called for dietic measures.

In the six hospitalized children the sedimentation rate (micro-ESR) (highest value) varied from 8 mm/hour to 31 mm/hour. The white cell counts on admission to the hospital varied from 6000 to 1[illegible],500 with a normal cell distribution.

During the observation period three of the members of the staff also developed acute respiratory illness. Two of them had a slight coryza without fever and a third one cough and fever.

TABLE 2. *Maximum temperature and duration of fever in 15 children with RS virus infection*

C	No. of cases	Fever (≥37.4)	No. of cases
< 37.3	1		
37.4–37.9	2	2–3 days	4
38.0–38.9	5	4–5	2
39.0–39.9	4	6–7	6
> 40.0	3	8–9	2
	15		14

Discussion

For the study of the epidemiology and the frequency of symptoms and clinical signs in different types of virus infections, it is in some respects more valuable to have the opportunity to investigate outbreaks of infections in closed groups (military camps, colleges, homes for children or families) than in hospitalized patients. Our investigation of an outbreak of RS virus infection in a home for infants is therefore of some interest. It gives information, for instance, on duration of RS virus excretion, incubation period and frequency of some clinical findings in RS infection.

The attack rate of RS virus in our study was 100% among the infants. A similar high attack rate has been reported by Kapikian *et al.* in institutional closed population [14].

The high frequency of RS virus isolation in our study is probably due to the fact that the specimens were inoculated into tissue culture immediately after being taken.

In measles and mumps as well as some other virus diseases, viruses can often be isolated a few days before onset of illness. This fact seems to be true also for RS virus infection. In our investigation RS virus was isolated during the period from days before to 9 days after onset of acute respiratory illness.

The incubation period was estimated to be 3–5 days which is in accordance with the report of Kapikian *et al.* [14].

The slow transmission of RS virus infection from case to case in this home for infants suggests that this infection may be considerably less contagious than varicella or morbilli. The transmission between the rooms may have been caused by some member of the staff. Two of them had a slight coryza and a third one coryza and fever during the investigation. However in none of the staff members studied, including the three mentioned above, could RS virus infection be established.

In the present material, infections with RS virus, as shown by virus isolations and rising antibody titres, were obviously well correlated in time with the occurrence of respiratory disease. As no other possible aetiology was found and, furthermore, RS virus has been associated with similar illness in other studies, the aetiological role of RS virus infection in our cases seems highly probable.

In two-thirds of the material the illness was diagnosed as febrile nasopharyngitis with cough, and in one-third the RS virus infection was associated with symptoms and signs from the lower parts of the respiratory tract. In the last-mentioned group both bronchiolitis and bronchopneumonia was seen. Three of the infants were critically ill but recovered. That RS virus infection sometimes can be fatal for infants has been reported [7, 12].

Summary

A report is given of an outbreak of acute respiratory illness in a home for infants in Stockholm. *Respiratory syncytial (RS) virus* was recovered from nasopharyngeal and/or throat swabs from 13 out of 15 infants (1–13 months old). Complement fixation (CF) tests against RS antigen showed at least 4-fold rises of antibody titre in 7 out of 15 paired sera, including sera from two infants, from whom no virus was isolated. There was no rise in CF antibody titre against adenovirus, influenza A and B, parainfluenza 1, 2 and 3, mumps or herpes simplex antigens. RS virus was recovered 2 days before the onset of illness as well as 9 days afterwards. The incubation period was estimated to be from 3 to 5 days.

All infants living in the home fell ill during a period from April 4 to April 30 1964. Two-thirds of the infants had febrile nasopharyngitis with cough and one-third had symptoms and signs from the lower parts of the respiratory tract. Two infants had bronchopneumonia and two others were critically ill in bronchiolitis. All infants survived.

References

1. Beem, M. O., Wright, F. H., Hamre, D., Egerer, R. and Oehme, M. Association of chimpanzee coryza agent with acute respiratory disease in children. *New Engl J Med, 263*: 523, 1960.
2. Beem, M., Egerer, R. and Anderson, J. Respiratory syncytial virus neutralizing antibodies in persons residing in Chicago, Illinois. *Pediatrics, 34*: 761, 1964.
3. Berkovich, S. and Taranko, L. Acute respiratory illness in the premature nursery associated with respiratory syncytial virus infections. *Pediatrics, 34*: 753, 1964.
4. Breton, A., Samaille, J., Gaudier, B., Gérard-Lefebvre and Ponté, C. Isolement du virus syncytial (virus C. C. A. de Morris) au cours de manifestations respiratoires bénignes épidémiques chez des prématurés. *Arch Franç Pédiat, 18*: 459, 1961.
5. Chanock, R. M. and Finberg, L. Recovery from infants with respiratory illness of a virus related to chimpanzee coryza agent

(CCA). II. Epidemiologic aspects of infection in infants and young children. *Amer J Hyg 66* 291, 1957
6. Chanock, R. M., Kim, H. W Vargosko, A. J Deleva, A., Johnson K. M., Cumming C. and Parrott, R. H.. Respiratory syncytial virus. I. Virus recovery and other observations during 1960 outbreak of bronchiolitis, pneumonia, and minor respiratory diseases in children. *JAMA 176* 647 1961.
7. Forbes, J A. Bennett N. McK and Gray N J Epidemic bronchiolitis caused by respiratory syncytial virus: clinical aspects. *Med J Aust, 2* 933, 1961.
8. Forsgren, M., Sterner, G and Wolontis, S.: Antibodies against respiratory viruses among healthy children. *Acta Pediat Scand 54* 214, 1965.
9. Fulton F and Dumbell, K. R.: The serological comparison of strains of influenza virus. *J Gen Microbiol 3*, 97 1949
10 Hilleman M. R., Hamparian V V Ketler, A., Reilly C M., McClelland L., Cornfeld, D and Stokes, J.: Acute respiratory illnesses among children and adults. *JAMA 180* 445, 1962.
11 Holzel, A., Parker, L., Patterson W H., White, L. L., Thompson K. M. and Tobin, J O'H.: The isolation of respiratory syncytial virus from children with acute respiratory disease. *Lancet 1* 295, 1962.
12. Holzel, A., Parker, L., Patterson, W H., Caldwell, D., White, L. L., Purdy R Thompson, K. M. and Tobin J O'H.: Virus isolations from throats of children admitted to hospital with respiratory and other diseases. Manchester 1962 1964. *Brit Med J 1* 614, 1965.
13. Johnson, K. M., Bloom, H. H., Mufson M. A. and Chanock, R. M.: Natural reinfection of adults by respiratory syncytial virus New *Engl J Med, 267* 68, 1962.
14. Kapikian A. Z., Bell, J A., Mastrota, R. J Johnson K. M., Huebner, R. J and Chanock, R. M. An outbreak of febrile illness and pneumonia associated with respiratory syncytial virus infection. *Amer J Hyg 74* 234, 1961
15. Lewis, F A., Rae, M L Lehmann, N I. and Ferris, A. A A syncytial virus associated with epidemic disease of the lower respiratory tract in infants and young children. *Med J Aust* 932, 1961
16. McClelland, L., Hilleman M. R., Hamparian V V., Ketler, A., Reilly C. M., Cornfeld, D. and Stokes, J Studies of acute respiratory illnesses caused by respiratory syncytial virus. *New Engl J Med, 264*. 1169, 1961
17 McClendon, J H. and Somers, F G Simple large scale ultrafiltration using osmotically forced dialysis. *Plant Physiol 30* 485, 1955.
18. Morris, J A., Blount R E. Jr., and Savage, R. E.: Recovery of cytopathogenic agent from chimpanzee with coryza. *Proc Soc Exp Biol Med, 92*. 544, 1956.
19 Moss, P D., Adams, M. O and Tobin, J O H Serological studies with respiratory syncytial virus. *Lancet, 1* 298, 1963.
20. Parrott, R. H., Vargosko A. J Kim, H. W., Cumming, C. Turner, H., Huebner, R. J and Chanock, R M.: Respiratory syncytial virus. II. Serological studies over 22-month period of children with bronchiolitis, pneumonia and minor respiratory diseases, *JAMA, 176* 653, 1961
21 Peacock, D B. and Clarke, S. K.: Respiratory syncytial virus in Britain. *Lancet, 2* 466, 1961
22. Sterner, G., de Hevesy G., Forsgren M., Tunevall, G and Wolontis, S.: Acute respiratory illness in children. A serological study with viral and bacterial antigens. *Acta Pediat Scand, 54* 226, 1965.
23. Svedmyr, A., Enders, J F and Holloway A.. Complement fixation with Brunhilde and Lansing poliomyelitis viruses propagated in tissue culture. *Proc Soc Exp Biol Med, 79* 296, 1952.
24 Ulstrup J C. Respirasjons-syncytie- virus (RS- virus), en årsak til alvorlige pneumonier hos spebarn. *Nord Med, 72* 1324, 1964.
25. von Zeipel, G and Svedmyr, A.: A study of the association of Echo viruses to aseptic meningitis. *Arch Ges Virusforsch, 7* 355, 1958.

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Acute Respiratory Illness with Mycoplasma Pneumoniae

An Outbreak in a Home for Children

by GÖRAN STERNER, GEORG DE HEVESY GÖSTA TUNEVALL and SIGVARD WOLONTIS

The role of Mycoplasma pneumoniae (MP) or Eaton agent in human respiratory illness has been extensively studied since 1957 [1 4, 5 6, 9 10 11 12, 14, 15 16 18] when Liu [13] reported that Eaton agent could be demonstrated in chick embryo lung by immunofluorescence. In Scandinavia MP infections have proved a common cause of primary atypical pneumonia (P.A.P.) in hospitalized adults [1 2, 10 18] The spread of MP is favoured in closed populations: families [10 11 15], military personnel [5 12] and pupils of boarding schools [8]

This report describes the first verified outbreak of MP infection in a home for children in Sweden.

Material and Methods

A girl, 11 years of age, was admitted to the Hospital for Infectious Diseases in Stockholm with a diagnosis of pneumonia on August 31 1964. Eight and eleven days later two other children (6 and 7 years old) were hospitalized for the same disease. These three children, members of the same family were living, for social reasons, in a home for children in Stockholm. In this home several other children fell ill with acute respiratory illness between September 1 and 9 An investigation was started on September 10 in order to elucidate the aetiology of this outbreak.

During the period of August 31 to October 22, twenty two children (1–12 years old) were living in the home (Table 1) Children below 3 years of age were housed in a separate ward. However the older children were allowed to visit the younger ones and some of the staff members worked in both parts of the home.

The children were clinically examined by one of us (G S.) on his visit to the home for taking blood samples and, when they fell ill by the pediatrician in charge of the home. The four hospitalized children were also examined by one of us (G S.)

The staff consisting of 15 members, was not included in this study

Cultures

Throat swabs for isolation of bacteria and MP were taken from 18 children on September 10 and from four hospitalized children on admission. The swabs were cultured on routine media for bacteria and on the modified PPLO medium described by Chanock [3] for MP

Enriching media for bacteria were not used and the occurrence of a few colonies of

TABLE 1 *Age distribution and the incidence of illness among 22 children living in a home for children in Stockholm during August 18–October 22 1964*

Age (years)	Total number	Thereof ill once	Thereof ill twice
1	7	4	3
2	1	1	
3	2	1	
4	2		1
5			
6	3	2	
7	1	1	
8	3	3	
9	1		
10			
11	1	1	
12	1		
Total	22	13	4

potentially pathogenic bacteria was not recorded as a positive finding.

Serology

T serum samples were obtained from 19 out of 22 children. The intervals between the blood samplings were from 20 days to 5 weeks. Paired sera were examined for complement fixing (CF) antibodies against adenovirus, influenza A and B, parainfluenza 1 2 and 3, respiratory syncytial (RS), mumps and psittacosis iruses as well as against commercial MP antigen (obtained from Robbin Laboratories, Inc., Chapel Hill, N.C., U.S.A.) These CF tests were carried out according to the method of Fulton & Dumbell [7] as modified by S ed myr *et al.* [20]. A four fold or greater rise of the antibody titre was considered as significant

Sera from 16 children were tested for antistreptolysin (AS), antistaphylolysin (ASta), antipneumolysin (APn) and for CF antibodies against Haemophilus influenzae (AHI). References for these reactions are given elsewhere [19]. A more than two-fold rise in antilysin reactions and four fold or greater rise in the CF test was considered as significant

Results

Mycoplasma and viruses

MP was isolated in four cases. As seen in Table _, ten children had a significant rise of CF titre against MP including one case from which MP was isolated. Four children had a CF titre of ≥16 against MP but without rise. In this group MP was recovered from two cases. In five cases both sera contained little or no CF antibody against MP (titres <4) and from none of them MP was isolated. From three children blood was not obtained but from one of them MP was isolated. Thus, proved or probable fresh infection with MP could be demonstrated in 15 out of 22 children.

TABLE 2. *The frequency of isolation of Mycoplasma pneumoniae (MP) n 22 children and the results of complement fixation (CF) tests against MP*

	Number of cases
Mycoplasma pneumoniae (MP) isolated from throat	4
CF antibody titre rise (four fold)	10*
CF antibody titre 16 but without rise	4
CF antibody titre < 4 in both sera	5
CF test not carried out	3*

Including one child from whom MP was isolated.
* Including wo children from whom MP was isolated.

TABLE 3 *Highest value of CF antibody titre (paired sera)*

Viruses	CF titre < 4	4	8	16	32	≥ 64	Number of cases with 4-fold rise
Adenovirus	3	3	3	4	3	2	1
Influenza							
A	19						
B	18		1				
RS virus	15		1	3			
Parainfluenza							
1	9	3	2	3	2		
2	17			1		1	
3	6		5	1	4	2	
Mumps	9			5	1	4	
Psittacosis	19						
Mycoplasma							
Mycoplasma pneumoniae	2	3		1	7	6	10

This case had also rising CF antibody titre against Mycoplasma pneumoniae.

On the other hand, as revealed in Table 3 antibody rise against a virus antigen (adenovirus) was found in one case only This was a boy 1 year old with a rising titre not only against MP and adenovirus but also against Haemophilus influenzae He had two febrile periods during the observation time It can also be seen from Table 3 that few or none of the children seemed to have had contact with parainfluenza 2 influenza A and B or psittacosis.

Bacteria

Only in one case were potentially pathogenic bacteria isolated, viz. Staphylococcus aureus. This child had no rise of ASta, however As mentioned above one child had a rise in AHI, two other children showed rises of APn.

Epidemiological data

The first case in this outbreak of MP infections was a girl of 11 years (Fig 1) She fell ill on August 18 with a pneumonia. Some of her friends outside the children's home had acute respiratory illness at that time and had probably infected this girl. The second case with acute respiratory disease appeared 12 days later on August 30 Unfortunately no serological studies were carried out in this case but MP was isolated. The next two cases with MP infection occurred on September 1 and 3 and they were followed, from September 6 to 9 by 12 cases of acute respiratory illness. At least seven of them were associated with fresh MP infection Again after a period of 12 days (September 21) another child developed acute respiratory illness associated with a MP infection.

As seen in Fig 1 four children had two episodes of illness during the observation period. One of them had, as mentioned above infections with three agents (adenovirus, MP and H. influenzae). The other three children had significant antibody rises against MP only Five of the 12 cases, developing acute respiratory illness during the period September 6 to 9 could not be definitely associated with MP infection.

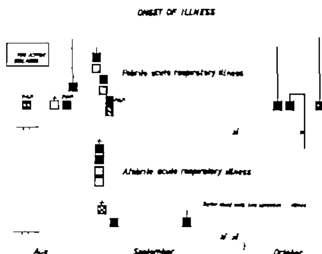


Fig. 1. Date for onset of illness. ■ Case with significant rise of CF titre against Myoplasma pneumoniae (MP). ▨ Case with CF titres > 16 against MP but without rise. ▦ Case with CF titres 4 against MP in both sera. ☐ Case not serologically studied. P.A.P primary atypical pneumonia.

In two of these cases a rise of APn was observed; one of them also had high CF titres against MP though without rise

The distribution in time of our cases conforms with previous observations of the incubation period of MP disease in volunteers who had been experimentally infected, i.e. 6 to 13 days (17)

Clinical observations

As seen in Fig 2 five children including two with MP infection were healthy

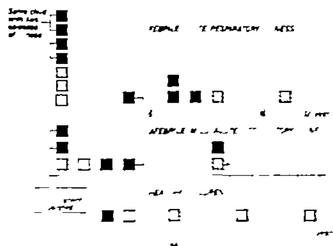


Fig. – Age distribution, incidence of Mycoplasma pneumoniae (MP) infection and disease in all 22 children in the home. Same symbols as in Fig. 1.

12. KINGSTON J R., CHANOCK, R. M., MUFSON M. A. HELLMAN L. P., JAMES, W D FOX, H. H. MANKO, M. A. and BOYERS, J Eaton agent pneumonia. *JAMA* *176*, 118, 1961
13. LIU C.: Studies on primary atypical pneumonia: I *Localization, isolation,* and cultivation of virus in chick embryos. *J Exp Med, 106:* 455, 1957
14. *LIU C., EATON M. D and HEYL, J T.:* Studies on primary atypical pneumonia: II. Observations concerning the development and immunological characteristics of anti body in patients. *J Exp Med, 109* 545, 1959
15. MARMION B P and HERS, J F The mycoplasma (PPLO) agents. Observations on Eaton primary typical pneumonia agent and analogous problems in animals. *Amer Rev Resp Dis, Conference on newer respiratory disease viruses, 88* part 2, 198, 1962.
16. NOTTEN M. C. J VAN and VEEN J VAN DER: Onderzoek op Mycoplasma pneumoniae ("Eaton agent). *Nederl T Geneesk, 107* 141 1963.
17 RIFKIND D CHANOCK, R. M. KRAVETZ, H. M., JOHNSON K. M. and KNIGHT, W Ear involvement (myringitis) and primary atypical pneumonia following inoculation of olunteers with Eaton agent. *Amer Rev Resp Dis, 85* 479 1962.
18. *STERNER, G SVEDMYR, A. TUNEVALL, G.* and W LOFTER, S. Infections with Eaton agent in pneumonia. *Acta Med Scand.* In press.
19 STERNER, G and TUNEVALL, G. Acute respiratory illness in children. A combined bacteriological and virological study *Acta Paediat Scand, 51* 349 1962.
20 SVEDMYR, A. ENDERS, J F and HOLLOWAY A.: Complement fixation with three types of poliomyelitis viruses propagated in tissue culture. *Amer J Hyg 57* 60, 1953.
21. TYRRELL, D A. J *Common Colds and Related Diseases.* Arnold Ltd., London, 1965.

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Experiences with Human Growth Hormone in Pituitary Dwarfism

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Growth hormone from human pituitary glands was first prepared by Gemzell & Heijkenskjöld [1] Li & Papkoff [4] and Raben [11]. Shortly afterwards Raben [1.] reported the successful use of human growth hormone (HGH) in the treatment of a boy with pituitary dwarfism. Since then the therapeutic value of HGH in this condition has been well established [8, 13 14, 16, 18, 19 20]. Because the hormone has to be prepared from human pituitary glands removed at necropsy each gland providing only 3 to 4 mg of HGH, ex perience with this form of therapy is still limited.

In the Pediatric Department, University Hospital, Oslo, we have used HGH in the treatment of pituitary dwarfs since 1961 So far twelve patients have been treated for periods varying from 6 to 41 months. Our experiences with these cases will be presented below Two patients treated less than six months will not be considered here

M thods

Human pituitary glands have been obtained at autopsy through the co-operat on of several pathologists. The glands ha been placed in a deepfreezer and sent to us in frozen state. HGH has been prepar l according to a modification of the m thod described by Roos, Fevold & Gemzell [15] The potency of the preparation has been determined in hypophysectomized infantile rats by the tibia test. A sample of the preparation has also been tested by Dr Greenwood, Imperial Cancer Research Fund, London. The immunological activity was found to be about 40 per cent higher than in the British Medical Research Council Standard A preparation.

Skeletal age was estimated by means of the atlas of Greulich & Pyle [2].

The metopirone (Su-4885) test was pe formed by the oral administration of 70–80 mg per kg body weight divided in 6–10 doses throughout 24 hours. The daily excretion of 17-hydroxycorticosteroids, 17 ketosteroids and the tetrahydro-S fraction was determined for one day before during and the day after metopirone administration.

Insulin sensitivity was studied by means of a combined insulin glucose tolerance test [17]. 0 1 I.U f crystalline insulin per kg body weight was given intra enously after an overnight fast. Thirty minutes later an oral dose of 1 75 g of glucose per kg body weight was given. Insulin tolerance was considered decreased, if there was con tinuous fall in blood sugar until glucose administration, i.e. not only during the first *0 minutes, but also from the 20 min to the 30 min sample.

TABLE 1 *Clinical and laboratory findings in the HGH-treated patients.*

Case No.	Sex	Age yrs./months	Height age, yrs./months	Skeletal age, yrs.	Insulin tolerance	Metopyrone response	PBI µg/100 ml	Cholesterol mg/100 ml	Phosphorus mg/100 ml
1	F	12/9	3/1	6	Decreased	Poor	4.6	280	4.2
2	M	9/5	1/10	1 3/12	Decreased	Poor	2.8	237	3.3
3	F	9/2	3/9	5	Decreased	Poor	4.3	302	4.0
4	F	16/4	9/0	10	Decreased	Poor	4 1	324	4.2
5	M	14/7	4/2	8	Decreased	Poor	4.0	193	3.8
6	M	8/6	2/0	4	Normal	Poor/Normal	3.6	268	3.7
7	F	19/6	10/9	12	Decreased	Normal	3.7	304	3.8
8	M	11/6	6/4	8	Decreased	Poor	6.6	232	3.8
9	M	7/11	3/10	5	Decreased	Poor	5.1	212	3.4
10	M	14/6	5/8	9	Normal	Normal	7.3	200	3.3
11	M	17/11	5/4	9 6/12	Decreased	Poor	3.7	304	3.8
12	M	17/2	10/0	10	Decreased	Poor	2.0	204	3.7

Material

Table 1 shows some clinical and laboratory data in the hypopituitary dwarfs treated with HGH. There were eight male and four female patients. All were severely growth retarded with marked retardation also in skeletal age. It will be seen that bone age in most cases was somewhat higher than height age The patients chronologic age at the beginning of HGH therapy ranged from 7 years 11 months to 19 years 6 months.

Insulin tolerance was found to be decreased in ten patients. The metopyrone response was normal in two, varying in one, and poor in nine patients. Thyroid function as judged by serum PBI and cholesterol seemed to be moderately or mildly impaired in nine patients.

In four patients (Nos. 1 2, 7 and 10) dwarfism no doubt was genetically determined. Three of these belong to the same family and have been described elsewhere [19]. The fourth (No. 10) is the product of a

TABLE 2 *Results of treatment with human growth hormone*

Case No.	Pre-treatment growth rate cm/year	Growth rate under treatment, cm/year 1st year	2nd year	3rd year	4th year	Additional therapy
1	1.5	11	8	5	3 cm/5 mos.	Thyroxine
2	1.5	13	8	6.5	1.5 cm/5 mos.[b]	Thyroxine
3	2	9	5.5	6	1.5 cm/3 mos.	Thyroxine
4	2	8	4.5	4[a]		Thyroxine
5	2	6	5			Thyroxine
6	1	7	3 cm/6 mos.			Thyroxine
7	2	8	2.5 cm/6 mos.			Thyroxine
8	3.3	7.5				Thyroxine
9	4	10.5	1 cm/1 mo.			Thyroxine
10	1.5	4 cm/7 mos.				
11	1.5	7.5 cm/7 mos.				Thyroxine
12	4	6 cm/7 mos.				Thyroxine

Thyroxine medication stopped for 9 mos owing to misunderstanding.

[b] Thyroxine and HGH dosage probably too low

consanguineous marriage, the parents being first cousins. Among his eight siblings two are severely dwarfed, an older brother now 31 years of age, and an older sister 19 years of age. It is of interest that these two siblings have gone through some degree of sexual maturation, however delayed and incomplete. This conforms with the opinion that sexual maturation is somewhat more frequent in the familial type of pituitary dwarfism than in sporadic cases [6]. Two other patients (Nos. 3 and 4) are also reported to have relatives of small stature, but these relatives have not been studied with respect to pituitary function.

In four cases (Nos. 5, 6, 9 and 11) there was history of traumatic birth and asphyxia in the neonatal period, which might have been of etiologic significance.

Most of the patients had not received any treatment for their dwarfism when HGH was started. A few of them had received thyroxine or anabolic steroids for short periods.

Birth weights were normal except in one patient, who was prematurely born. Reliable information concerning growth during the first year of life, with height measurements, was available for six of the twelve patients. *All these six showed definite growth retardation, either moderate or marked, even during the first year of life.* It is our firm belief that most hypopituitary dwarfs, contrary to the statements in many textbooks, are found to be growth retarded before one year of age when closely studied.

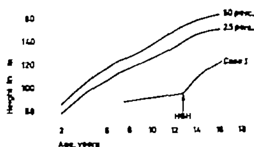


Fig. 1 Response to HGH therapy through 41 months in case 1. Arrow indicates start of treatment.

Treatment and Results

The usual dosage has been 2 mg HGH intramuscularly three times a week for patients with body weights up to 25 or 30 kg, 3 mg three times a week for the heavier patients. Higher doses have not been used. All patients except one have received additional thyroxine therapy usually 0.1 mg daily. No other medication has been given.

The importance of giving thyroxine in order to obtain optimal results with HGH in cases where signs of secondary hypothyroidism are present is illustrated by our case No. 4. During her third year of treatment thyroxine medication was by misunderstanding stopped for nine months. In this period her height increment was *only 1.5 cm. When thyroxine 0.1 mg daily* was once more combined with HGH she grew 3.5 cm in three months.

Side effects of HGH administration were few and insignificant. During the first days of treatment most patients complained of some nausea, which was easily controlled by symptomatic medication. After the first week nausea was not complained of any more. A decrease in the amount of subcutaneous fat was seen in most cases. Pitting oedema occurred in one patient (No. 5) during HGH therapy. Under continued treatment the oedema disappeared within three months.

The results obtained with HGH appear in Table 2. The pre-treatment growth rate ranged from 1 cm/yr to 4 cm/yr. During the first year of treatment height increments varied from 6 cm to 13 cm, with an average of 10 cm. This higher-than-normal rate of growth in the beginning of HGH

therapy has also been the experience of others and is considered to be the so-called catch up growth [9].

The five patients treated for a full second year gained on an average slightly above 6 cm in height, with individual variations from 4.5 to 8 cm. This means that a normal rate of growth has been achieved.

The three patients treated with adequate doses also for a third year grew from 5 to 6.5 cm.

Our first two patients have been treated for 3½ years. They have grown 27 and 29 cm respectively Patient No 1 still has a normal growth rate (Fig 1) In case 2 growth has been less satisfactory during the last six months, probably due to underdosage During the whole period of treatment he has received 2 mg HGH three times a week and 0 1 mg of thyroxine daily

Our oldest patient (No 7) has now reached the height of 153.5 cm, i.e 2.5 cm above the 2.5 percentile and the second oldest (No 4) has almost reached the 2.5 percentile

Discussion

The diagnosis, pituitary dwarfism, may be difficult to establish with absolute certainty before adult age. In the present study it was based mainly on the following data All twelve treated patients were markedly or severely dwarfed with marked delay in skeletal maturation. Otherwise the most important diagnostic findings were increased insulin sensitivity (10 cases) a poor response to metopirone administration (9 possibly 10 cases) signs of secondary hypothyroidism (9 cases) and a falling or poor sexual maturation after the normal age of puberty

The fact that all the patients responded well to long term administration of human growth hormone at a dose level which is probably within or below but certainly not above the range of physiological replacement therapy [8] also indicates that the diagnoses have been correct. Most workers have found that such doses do not significantly promote growth in other forms of dwarfism at least not to the same extent as in our patients. However Raben *et al* [14] have reported a slight and probably only transient growth-promoting effect of HGH in similar dosage in a few patients with assumed constitutional dwarfism. This observation needs further confirmation.

We find it useful to test insulin sensitivity by an intravenous insulin load followed by oral glucose administration thirty minutes later [17] When performing metopirone tests in children we think it is an advantage to determine the urinary excretion not only of 17 hydroxy steroids, but also of the tetrahydro-S fraction, because the basal excretion of this fraction is so minute and the rise following metopirone administration, therefore, more pronounced in individuals with a normal ACTH reserve

When the newer radio-immuno-assay for HGH in plasma [3] becomes more readily available it will probably be of great diagnostic value in cases where the results of our previous, indirect diagnostic methods leave some doubt about the diagnosis.

The etiology of pituitary dwarfism is varying and often entirely unknown in the individual case Traumatic birth with

asphyxia in the neonatal period is probably one of the most frequent etiologic factors. In four of our patients the history indicates this cause. The presented material shows that genetically determined pituitary dwarfism with autosomal recessive mode of transmission is not extremely rare, at least in Norway. We have previously reported a family with eight dwarfed members through five generations [19]. Three of these are included in the present material. In another unrelated, family three siblings are affected, one of whom is included in this report. We have also seen several other hypopituitary dwarfs (two of them included here) in whose families there have been other members with stunted growth. However the pituitary function of these family members has not been studied, and the importance of this observation is uncertain. It might be mentioned in this connection that in the Pediatric Department in Bergen, western Norway two siblings have been treated for pituitary dwarfism [5]. None of the patients in the present material had craniopharyngeoma.

The results of HGH therapy in our series are at least as good as those reported by others [8, 13 16, 20], even though we have tried to keep the HGH dosage at a low level in order to be able to help as many patients as possible with our limited supply. It is not unlikely that somewhat better height increments could have been obtained by means of higher doses.

In accordance with others we have achieved the greatest gain in height during the first year of treatment, on an average 10 cm. Many patients showed a greater than-normal growth rate during this period of time ("catch-up" growth). After the first year the rate of growth of the patients has been normal, usually about 6 cm/year

Prader *et al* [10] have reported that three out of nine HGH treated pituitary dwarfs rapidly developed resistance to HGH therapy due to the formation of specific antibodies in high titers. Parker *et al.* [7] observed that one of their thirteen patients became resistant after seven months treatment for the same reason. The formation of growth hormone antibodies in amounts that do not interfere with the clinical effect of the hormone seems to be a rather common occurrence under prolonged treatment.

None of our patients has so far become unresponsive to our usual doses of HGH. Whether this simply is due to chance, or our growth hormone preparation has certain advantages in this respect over that used by Prader *et al* [10] and Parker *et al* [7], is an open question. It is possible that the Raben procedure with extraction in glacial acetic acid and heating to 70°C may denaturate the hormone to a greater extent, making it more antigenic, than the method used by us [15]. With this method the material is subjected only to aqueous solutions of near neutral pH and all steps performed at 0–4 C. This point deserves further investigation. The two siblings treated for 3½ years have no HGH antibodies (Dr N Norman, Aker Hospital, Oslo) but we have not had the opportunity of studying the presence of HGH antibodies in our other patients.

The most serious objection to the use of testosterone and anabolic steroids to promote growth in hypopituitary dwarfs is that these drugs accelerate skeletal maturation more than growth and thereby

hasten epiphysial fusion. The growth response to HGH treatment has not been accompanied by a disproportionate acceleration in bone age in any of the patients in our series. Others have reported similar experiences [16, 20].

Perhaps the most important question is whether a final height can be achieved with HGH treatment of hypopituitary dwarfs which is within accepted limits of normality. Our experience indicates quite strongly that a height within the normal range (i.e. above the 2.5 percentile) can be expected in the more moderate cases, even if therapy is started rather late. Whether as good end results can be obtained in the most severe cases is not yet possible to tell. In these patients treatment should probably be started as early as possible, and the chances of obtaining normal height will then depend on the extent to *which the development of HGH anti*bodies can be avoided.

When an acceptable height has been achieved by means of HGH in these *patients, the problem of sexual maturation* must be considered. Some patients will spontaneously mature sexually at least to some extent, but the great majority will need substitutional therapy. The common practice is to give gonadal hormones in order to produce more or less satisfactory secondary sex characteristics. But fertility can of course not be achieved by this means. Theoretically it should be possible to produce a more complete sexual maturation and perhaps fertility by means of human pituitary gonadotrophic hormones, and this possibility deserves further investigation. The great practical disadvantage of such therapeutic trials would be that frequent injections through many years would have to be given. An alternative approach could be to start substitution with gonadal hormones and consider the use of human gonadotrophic hormones later if the desire to obtain fertility arises as a practical problem for the patient.

Summary

Experiences with HGH treatment of twelve hypopituitary dwarfs for periods varying from 6 to 41 months are reported.

All the patients were considered to have hypopituitarism from birth: four of them of the hereditary type, four possibly due to traumatic birth or neonatal asphyxia, and four from an unknown cause. In six patients detailed information concerning growth during the first year of life could be obtained. All of these patients had a *degree of growth retardation before one* year of age. We consider this to be the rule rather than the exception in pituitary dwarfism.

The most important diagnostic evidence was a marked degree of growth retardation accompanied by a pronounced delay in skeletal maturation, and, in most cases, *signs of secondary hypothyroidism*, increased insulin sensitivity and a poor response to metopirone administration. None of the patients who had passed the normal age of puberty had matured sexually.

All twelve patients responded well to HGH therapy. During the first year of treatment the average height increment was 10 cm ("catch-up" growth). With continued treatment a normal rate of growth, about 6 cm/year, was obtained. There was no disproportionate accelera-

tion of skeletal maturation in any of the patients. None of them has so far become resistant to HGH administration. The oldest patient has reached normal height. Whether this will be possible not only in the moderately severe, but also in the most severe cases of pituitary dwarfism, can not yet be stated.

The possibility of using human gonadotrophic hormones in an attempt to produce sexual maturation in these patients is discussed briefly.

References

1. Gemzell, C. A. and Heijkenskjöld, F. Growth hormone concentration in human pituitaries. *Endocrinology, 59:* 681, 1956.
2. Greulich, W. W. and Pyle, S. I.: *Radiographic Atlas of Skeletal Development of the Hand and Wrist.* 2nd ed., p. 256. Stanford University Press, Stanford, California, 1959.
3. Hunter, W. M. and Greenwood, F. C.: A radio-immuno-electrophoretic assay for human growth hormone. *Biochem J. 85:* 39, 1962.
4. Li, C. H. and Papkoff, H.: Preparation and properties of growth hormone from human and monkey pituitary glands. *Science, 124* 1293, 1956.
5. Mos, P. J. Personal communication.
6. Nowakowski, H. and Lenz, W. Genetic aspects in male hypogonadism. *Recent Progr Hormone Res, 17:* 53, 1961.
7. Parker, M. L., Mariz, I. K. and Daughaday, W. H.: Resistance to human growth hormone in pituitary dwarfism: clinical and immunological studies. *J Clin Endocr 24,* 997, 1964.
8. Prader, A., Illig, R., Széky, J. and Wagner, H.: The effect of human growth hormone in hypopituitary dwarfism. *Arch Dis Childh, 39:* 535, 1964.
9. Prader, A., Tanner, J. M. and von Harnack, G. A.: Catch-up growth following illness or starvation. *J Pediat, 62.* 646, 1963.
10. Prader, A., Wagner, H., Széky, J. Illig, R., Tanner, J. L. and Maddaiah, D. Acquired resistance to human growth hormone caused by specific antibodies. *Lancet, II:* 378, 1964.
11. Raben, M. S. Preparation of growth hormone from pituitaries of man and monkey. *Science, 125:* 883, 1957.
12. — Treatment of a pituitary dwarf with human growth hormone. *J Clin Endocr 18:* 901, 1958.
13. — Growth hormone, 2. Clinical use of human growth hormone. *New Engl J Med, 266:* 82, 1962.
14. Raben, M. S., Matsuzaki, F. and Minton, P. R.: Growth-promoting and metabolic effects of growth hormone. *Metabolism, 13:* 1102, 1964.
15. Roos, P. Fevold, R. and Gemzell, C. A. Preparation of human growth hormone by gel filtration. *Biochem biophys Acta* (Amst.), *74:* 525, 1963.
16. Soyka, L. F., Ziskind, A. and Crawford, J. D.: Treatment of short stature in children and adolescents with human growth hormone (Raben). *New Engl J Med, 271:* 54, 1964.
17. Tryostad, O. The insulin-glucose tolerance test in pituitary growth retardation. *Arch Dis Childh, 40:* 508, 1965.
18. Tryostad, O. and Skip, M. Hypofysær vekstretardasjon. Erfaringer med somatotropinbehandling ed hypofysær nanisme. *T norske Lægeforen, 83:* 1443, 1963.
19. — Hereditary pituitary dwarfism treated with human growth hormone. *Acta Paediat Scand, 53:* 527, 1964.
20. Wright, J. C. Brasel, J. A., Aceto, T. Finkelstein, J. W. Kenny, F. M., Spaulding, J. S. and Blizzard, R. M.: Studies with human growth hormone (HGH). *Amer J Med, 38:* 499, 1965.

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The Oxygen Cost of Minor Changes in Heat Balance of Small Newborn Infants

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One of the central difficulties in physiologic studies of the neonate stems from lack of agreement concerning the thermal conditions which must be provided to insure that measurements are made when the infant is in a resting (or "basal") state. Although several studies [4, 11 15] have demonstrated that 32–34°C ambient air temperature appears to be "neutral" for the newborn infant, it is unlikely that this condition will be generally applicable since it is probably restricted to a fairly narrow range of wind speed, humidity and radiative exchange. We demonstrated [18] that under commonly encountered physical conditions these air temperatures constituted heat losing environments which were beyond the cold limits of some very small naked infants. Thus there is no accord on whether the "neutral state" should be described in terms of ambient thermal conditions or body temperature (*s*) moreover the distinction is often not made between acute responses and steady-state relationships of metabolism and (body or ambient) temperature

The present investigation sought to obtain data which would be useful in providing answers to some of these questions. We have estimated the oxygen cost of modest changes in heat balance of small newborn infants in an effort to define the "neutral body temperature" of these babies.

Subjects and Methods

The subjects of this study were 24 small newborn infants who were selected as asymptomatic "normal" representatives of the general population of light infants (birth weight 1001–1500 g) admitted to the Neonatal Unit of Columbia Presbyterian Medical Center in 1963 and 1964. Most of the babies were girls (17/24) about a third (7/23) were markedly undergrown (birth weight < tenth percentile of the Colorado standard [14]) and almost all (20/23) were born prematurely (< thirty-seventh week of gestation) (Fig. 1). Four twins were studied

Measurements were made in servo-controlled, Electropane-heated incubators pre

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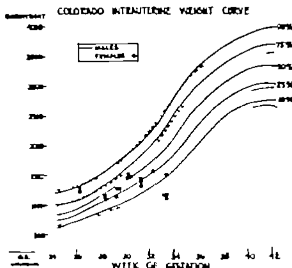


Fig. 1. Birth weight, gestational age and sex of the study population compared to the Colorado standard [14].

viously described by us [1]: moderately humidified room air (35 R.H.) flowed through the incubators at rat of 35 liters per minute. At ages ranging from 1 to 3 days each infant was undressed completely and placed supine in an incubator; the warming system was adjusted to regulate exposed anterior abdominal skin temperature at one of three levels under test, 35 36 or 37°C. The prescribed condition (±0.1°C of the set point) was maintained overnight (range 15–25 hours) to assure complete and stable thermal equilibration. Care and feeding (simulated breast milk) proceeded in the usual manner during this period (including intravenous infusion of 10% dextrose in 5 normally grown and 2 undergrown infants). The physiologic measurements were made early the next morning one-half t one hour after a feeding. Skin temperatures, T (forehead, bdomen, upper arm, dorsum of hand, thigh, dorsum of foot, and interscapular area) colonic temperature T (ca. 17 cm from the anus), incubator temperatures, T (inner wall of incubator and air ca. 10 cm above the infant), and room temperature T_{w} were all sensed with thermistor probes (Yellow Springs Instrument Co. 400 series) and registered on a Yellow Springs Tele-thermometer The resting respiratory frequency was counted and expired air was then collected in recording spirometer by means of a low dead space (0.75 ml) nasal coupler; the pressure-flow characteristics of the collection system (Fig 2) were similar to those described by Golinko & Rudolph [9]. After ppropriate wash out of the dead space of the collecting system, replicate timed collections of expired air (≅5 min) were obtained, and body and ambient temperatures were again noted. If the infant rested quietly or slept during the collections the runs were accepted as satisfactory The volume of expired air was corrected to standard conditions (STPD) and the fractional concentrations of respiratory gases were analyzed with a Fisher Hamilton Gas Partitioner for the computation of minut oxygen consumption ($\dot{V}_{O_2}$), carbon dioxide production ($\dot{V}_{CO_2}$) and respiratory exchange ratio (R) using the usual respiratory gas equations. Gaseous metabolism was expressed as absolute oxygen consumption (i.e. ml oxygen consumed per minut per *fant*) instead of being referred to some unit of body size. This convention was

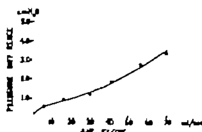


Fig. 2. Resistance to continuous air flow through the respiratory valve and spirometer

adopted because there was considerable between-infant variation in the intensity of metabolism in this group of infants of fairly uniform weight; not surprisingly there was marked dispersion of gestational ages (range 13 weeks). As will be discussed elsewhere [17] much of the variation in oxygen consumption may be accounted for by differences in body composition, particularly the proportion of active protoplasmic mass per unit of total body weight [2], among infants whose antenatal growth was markedly different.

Arterial (or arterialized capillary) blood was obtained for determination of acid base status and oxygen saturation by the methods of Siggaard-Andersen [16] t the conclusion of the respiratory measurements. If evidence of hypercapnia (pCO >45 mm Hg) or hypo emia (Hbo_2 sat. <80%) was uncovered, infant were excluded from the study.

Following completion of the first test condition the feed-back warming system was adjusted to control abdominal skin temperature at the second level, and the above noted measurements were repeated after overnight equilibration. An identical procedure was followed for the third and final test condition. The order of exposure of each infant to the three test conditions ($T_{s(abd)}$ 35 36 and 37°C) was randomized by means of the Latin Square strategy (Table 1) to insure equality of test order (35°C first—8 infants, 35°C second—8 infants, 35°C third—8 infants 37°C third—8 infants) and to permit analysis of differences in which each infant served as his own control.

Average skin temperature ($T_{\bar{s}}$) was computed with weighting factors derived from Klein & Scammon [13] measurements of surface area in human fetuses (forehead .23 abdomen .33, upper arm 11 thigh .23 hands 06, feet 05); weighted average total body temperature ($T_{\bar{B}}$) was calculated on the assumption that the core (colon) represents 6, the skin 4 of the total. Metabolic ratios (compared with $T_{s(abd)}$ 36°C condition) were expressed on the basis of change in a erage body temperature as follows

$$\frac{\Delta V_{O_2}}{\Delta T_{\bar{B}}} + V_{O_2} \quad T_{s(abd)}\ 36\ C \quad 100$$

Results

When the abdominal skin temperature of small infants in this study was regulated at 35° 36° and 37°C by means of an exogenous heat source, the means of the total

Table 1 *Latin square order-of-exposure assignment table*

Infant	Order of Exposure First	Second	Third	Infant	Order of Exposure First	Second	Third
A	35	36	37	D	36	35	37
B	36	37	35	E	35	37	36
C	37	35	36	F	37	36	35

The order of assignment for the remaining 18 infants was determined by 3 similar Latin square tables; 6 infants in each table.

TABLE 2. *Body temperatures (°C) and gradients in three thermal conditions.*

Body temperatures and gradients	Abdominal skin control temperature 35	36	37
T_S			
Mean	34.8	35.8	36.8
S.D.	±0.26	±0.71	±0.34
S.E.	±0.06	±0.14	±0.07
T			
Mean	35.2	36.2	37.0
S.D.	±0.70	±0.44	±0.42
S.E.	±0.14	±0.09	±0.08
$T_c - T_S$			
Mean	1.0	0.5	0.6
S.D.	±0.51	±0.34	±0.36
S.E.	±0.10	±0.07	±0.07
$T_c - T$ (foot)			
Mean	2.8	2.1	1.3
S.D.	±1.47	1.24	±1.07
E.	±0.30	0.25	±0.22

body temperatures (T_S) at complete equilibration were found to be 34.8°, 35.8° and 36.8°C respectively. Although there was evidence that these infants were able to alter the deep-to-superficial thermal gradients ($T - T_S$) they were generally unable to maintain a dissociated deep body temperature (T) (Table 2).

Mean oxygen consumption was lowest in the $T_{(abd)}$ 36°C condition (7.9 ml *per kg*/min., S.E. ±0.25). The most frequent result of equilibration at the lower and higher thermal levels was an increase in metabolic rate. In the $T_{(abd)}$ 35 C condition the mean increase was 10.9 % for 1 C fall T_S (S.E. ±4.08 %), a change that was greater than would reasonably be expected to occur by chance ($t = 2.67$ $d.f. = 23$, $p < 0.02$). The oxygen cost at a warmer level ($T_{(abd)}$ 37°C) was smaller (5.9 % for 1°C increase T_S, S.E. ±3.51) and the rise in oxygen consumption might more easily be explained by chance ($T = 1.60$ $d.f. = 23$ $p < 0.20$).

Other Observations

The mean respiratory exchange ratio in 17 infants who were not receiving dextrose infusions, was lowest in the cool condition ($T_{(abd)}$ 35°C, $R = 0.87 \pm$ S.E. 0.031) and showed a small upward trend in the two warmer conditions ($T_{(abd)}$ 36 C $R = 0.88 \pm 0.016$; $T_{(abd)}$ 37°C, $R = 0.89 \pm 0.066$). The ratios were generally highest in the 7 infants receiving I.V. dextrose.

As can be seen in Fig. 1 the seven undergrown infants in this study were not only markedly smaller at birth than expected for gestational age, but they were also generally lighter than the remaining 16 infants whose gestational ages were known. On equilibration in slightly cool conditions ($T_{(abd)}$ 35°) these small infants of advanced gestational age generally maintained a somewhat higher rate of metabolism and a larger temperature gradient between deep and superficial tissues than their normally grown coevals. However there was too much individual variation, the two groups were not evenly matched with respect to order-of-exposure and the numbers of infants involved were too small to permit a formal comparison of the responses of the normally grown and undergrown infants in this study.

Discussion

The relationship between ambient thermal conditions and gaseous metabolism

in human neonates has been studied extensively in the past few years and there is general agreement that these infants respond with prompt and appropriate changes in oxygen consumption rate when there are changes in the thermal demand of the environment. Most measurements have been made after a relatively short exposure to various test environmental conditions (usually <2 hours). These observations have given a clear indication of newborn infants acute responses during cooling and warming, but the relationships may be quantitatively and qualitatively different when heat production and heat loss are finally balanced after a sufficiently long stabilization time in a given physical environment. For example Hsieh [12] observed that rats living in cool environments had an elevated metabolism which fell when the animals were transferred to a warm room If insufficient time (<2½ hours) was allowed for the rats to reach a steady state in the new environment, estimations of gen consumption were too high. The results of other studies in animals [3 10] of ambient temperature and metabolism also suggest that the time factor must be given due consideration in describing the relationships. This influence has not been considered in most investigations of body temperature and metabolism in the human neonate and as a consequence it is difficult to extrapolate from these results to the steady-state condition. Moreover there continues to be little agreement on which regional temperature in the body should be used to make the comparisons, for as Du Bois emphasized long ago [8] there is no uniformity of temperature within the human body

In the present study we made a special effort to balance heat exchange between the infant and his environment so that superficial body temperature (anterior abdominal skin) would be stabilized. This stratagem was chosen to permit evaluation of the relationship between a fixed level of body temperature and metabolism, removing the effects of between-infant variations in body temperature [18] Surface temperature was selected as the control site because there is evidence [4, 19] *that in neonates relatively small* changes in the temperature of the skin are consistently followed by changes in the rate of oxygen consumption. Moreover the exposure periods were prolonged to insure that the metabolic measurements were made *well after the establishment of a* thermal steady state.

We interpret the results as indicating that a 'neutral thermal state was achieved in the small asymptomatic subjects in the present study when environmental conditions were adjusted to maintain a stable abdominal skin temperature close to 36°C. When heat loss was increased and superficial temperature (T_{abd}) controlled at 35°C, the infants exhibited qualitatively appropriate homeothermic responses (e.g. increased oxygen consumption and increased deep-to-superficial temperature gradients) but the magnitude of these responses was generally inadequate to prevent a fall in deep body temperature.

Although the average increase in oxygen consumption which occurred when the abdominal skin was stabilized at 37°C was somewhat equivocal and does not inspire great confidence, we are tempted to speculate about the magnitude of the change.

The increase in metabolism which occurs when homeotherms are warmed beyond the thermoneutral point is related to the Van't Hoff effect (i.e. the heat production of living cells will increase two or three times if their temperature is raised by 10°C). Thus if one assumes that for most metabolic processes the Q10 is between *.0 and 3.0 a rise in average body temperature of 1°C beyond thermoneutrality should be associated with an increase in oxygen consumption between 7.2 and 11.6%. Christensen [7] noted a 10.8% rise in oxygen consumption in adults for 1°C increase in body temperature raised by diathermy He estimated that 1– *" of this increase was due to elevated metabolism in heart lung and viscera and attributed the remainder to an increase in general cellular reactions. We suspect that the relatively small average increase observed in the present study (5.9 ,.) may indicate that the thermostatic set point in these infants was slightly higher than the arbitrarily chosen control level of $T_{(skin)}$ 36°C

The mean deep body temperature of small infants in the present study when in a presumed neutral thermal state is lower (T 36.2 C) than that expected in larger neonates. This finding is in agreement with the observations of Brück and co-workers [5] who observed in acute experiments that when environmental conditions were adjusted to promote minimal oxygen consumption, the deep rectal temperatures ranged between 35 to 36.5 C* in infants with body weights below 1500 g (1270–1480) larger infants (1540–.530 g) had higher deep rectal temperatures (36.5–37.5 C) Brück *et al* [5] concluded that the set point of the deep body temperature in very small babies was below the range which is generally considered to be a normal central body temperature in man. Our results in infants exposed for more prolonged periods tend to support this view however we are not convinced that the "set point" of the peripheral thermoreceptors is similarly turned down since in the neutral state the skin temperatures of small infants in the present study were relatively close to those observed in large neonates [4]. We suggest that this may be taken as indirect evidence for the view that superficial and not deep thermoreceptors are exerting the dominant influence over metabolism in the neonate. Skin temperature should be regularly noted in physiologic studies of newborn infants and we propose that measurement of the temperature of exposed anterior abdominal skin be considered for routine clinical thermometry

In a previous study [18] we noted that small newborn infants (<1500 g) of advanced gestational age were relatively successful in minimizing the fall of deep body temperature when exposed for 2–8 hours to a cool environment as compared with infants fo comparable size born after shorter periods of gestation. In the present study there was a similar indication in more prolonged exposure to slightly cool conditions, but as already stated a firm conclusion on this point is not possible. In future studies of temperature and metabolism in neonates we recommend that the experimental design be devised to permit a confident comparison of undergrown and normally grown subjects.

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* The skin temperatures of these small infants as not reported

Summary

Measurements of gaseous metabolism in small newborn infants whose anterior abdominal skin temperatures were stabl lized at fixed levels (35 36 and 37°C) for 15 to 25 hours revealed that mean oxygen consumption was lowest in the 36°C condition. From the oxygen cost of small departures in heat balance it is suggested that a "neutral thermal state in small neonates may be achieved when environmental conditions are adjusted to maintain the abdominal skin temperature between 36 and 37°C. The deep body temperature in the neutral thermal state is lower in these very small babies than is generally considered to be a normal deep body temperature in human subjects.

The relationship between temperature and metabolism in undergrown as compared with normally grown infants should be investigated further

References

1. Ao te, F J J ., and Silverman W A.: The control of body temperature in the small newborn infant by low-energy infrared radiation. *Pediatrics, 31* 725, 1963.
2. Benedict F G and Talbot, F B.: *The Gaseous Metabolism of I fants.* Carnegie Institution Reports, Washington, 1914.
3. Blatteis, C. M.: Hypoxia and the metabolic response to cold in newborn rabbits. *J Physiol, 172* 3-8 1964.
4. Bruck, K.: Temperature regulation in the newborn infant. *Biol Neonat, 3* 65, 1961
5. Bruck, K. Parmelee, A. H., J and Bruck, M Neutral temperature range and range of thermal comfort in premature infants. *Biol Neonat 4* 32, 1962.
Burton A. C. and Edholm, O G *M in Cold Environment* London, Edw Arnold. 1955.
7. Christen et E. H.: Beitrage zur Physiologie schwerer korperlicher Arbeit. IX Hyperthermie und Rubestoffwechsel. *A beitsphysiologie 7* 120 1933.
8. Du Bois, E. G *Lane Medical Lectures. The Mechanism of Heat Loss and Temperature Regulation.* Stanford Uni Press, Stanford University Calif., 1937
9. Golinko, R. J and Rudolph, A. M.: A valve for respiratory studies in infants. *Pediatrics 27* 645, 1961
10. Hahn, P Koldovsky O., Krecek J Martinek, J and Vacek, Z.: Endocrine and metabolic aspects of the development of homeothermy in the at. In *Somatic Stability in the Newly Born.* Ciba Foundation Symposium, Boston, Little, Brown and Co., 1961
11. Hill, J R.: The development of thermal stability in the newborn baby In *The Adaptation of the Newborn I fant to Extra Uterine Life.* Nutricia Symposium. H. E. Stenfert Kroese N V Leiden, 1964
12. Hart, A. L.: The basal metabolic rate of cold-adapted rats. *J Physiol, 169* 851 1963.
13. Klein A. D and Scammon R. E.: Surface area and age in prenatal life. *Proc Soc Exp Biol Med, 27* 461 1940.
14. Lubchenco L. O Hansman C., Dressler, M., and Bo d E.: Intrauterine growth as estimated from live-born birth- eight data at 24 to 42 weeks of gestation. *Pediatrics, 32* 793 1963.
15. Oliver, T K., J and Karlberg P Gaseous metabolism in newly born human infants. *Am J Dis Child 105,* 427 1963.
16. Siggaard-Andersen O *The Acid-Base Status of the Blood.* Williams and Wilkins, Baltimore, 1964
17. Sinclair, J C., Scopes, J W and Silverman W A. Metabolic reference standards in the neonate. (In preparation.)
18. Silverman, W A. and Agate, F J J Variation in cold resistance among small newborn infants. *Biol Neonat 6,* 113, 1964.

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Antibody Response after Immunization with Typhoid Paratyphoid A and B Vaccine in Kala-azar

by CHRISTOS CASSIMOS, STELLIOS LAZANAKIS and THEODORE THOMAIDIS

An impairment of antibody production has been noted in diseases of the lymphoreticular system such as sarcoidosis [6], myeloma [7, 8] and malignant lymphomas [5] in which an abnormal γ-globulin is present.

The present study was undertaken in order to investigate antibody production in Kala-azar, which is also a disease of the reticuloendothelial system. This condition is accompanied by altered serum proteins [10] and bears great similarities to the aforementioned conditions except for the fact that it is a curable disease of known etiology.

Material and Method

Twelve patients with Kala-azar of ages varying from 1 year to 12 years were selected at random from the wards of our clinic, and twelve healthy individuals of comparable ages were used as controls for the present study. The patients presented the typical features of Kala-azar, i.e. fever, hepatosplenomegaly, anemia, leucopenia, decreased serum albumin and increased γ-globulin by electrophoresis. The diagnosis of Kala-azar was established upon the finding of *Leishmania* in bone marrow smears. Both patients and controls were given a total of three subcutaneous injections of T.A.B. suspension at weekly intervals. T.A.B. suspension was prepared in the laboratories of the Greek Public Health Service and contained per ml 1 000,000 Salmonella typhosa, 500,000,000 Salmonella paratyphi A and 500,000,000 Salmonella paratyphi B organisms. Doses used for each injection were 0.2 ml for infants, 0.3 ml for children under 5 years and 0.5 ml for older children.

Sera obtained prior to immunization, one week after the 2nd injection and one week after the 3rd injection were studied by the rapid slide method [3] for agglutinin activity to T.A.B. and its titer. Antigens used were the Lederlee product of O antigen of Salmonella typhosa and H antigen of all three organisms.

Tests were performed as follows: 0.04 ml, 0.02 ml, 0.01 ml and 0.005 ml of serum were pipetted successively and placed on four clean slides. This represents the equivalent of serial dilutions of serum in the order of 1/40, 1/80, 1/60 and 1/320 respectively. Then one drop of antigen was added to each of these slides. The slide was mixed gently and observed for precipitation. Titer was expressed by the denominator of the dilution which was the last to show precipitation.

Abbreviations: T.A.B., Typhoid Paratyphoid A and B vaccine; *Anti-O* antibody against somatic antigen of the Salmonella group D (Typhoid O); *Anti TH* antibody against Typhoid H antigen (flagellar); *Anti-AH* antibody against Paratyphoid A antigen (flagellar); *Anti BH* antibody against Paratyphoid B antigen (flagellar).

TABLE 1. *Titers of anti-O and anti-H antibodies one week after the 2nd and one week after the 3rd immunizing dose of T.A.B vaccine in 12 controls*

		Titers of antibodies one week after the 2nd dose of T.A.B. vaccine				Titers of antibodies one week after the 3rd dose of T.A.B vaccine			
Case N	Age	Anti TO	Anti TH	Anti-AH	Anti BH	Anti TO	Anti TH	Anti AH	Anti BH
1	12 mo	—	> 320	160	40	—	> 320	160	80
2	14 mo	—	> 320	160	80	—	> 320	> 320	160
3	17 mo	80	> 320	40	40	80	> 320	80	80
4	18 mo	—	> 320	—	40	—	> 320	40	40
5	23 mo	—	> 320	160	160	40	> 320	160	160
6	3 yrs	—	> 320	160	80	—	> 320	160	160
7	3 yrs	—	160	80	—	—	160	80	40
8	3 yrs	—	> 320	40	40	—	> 320	80	40
9	4 yrs	—	> 320	160	80	—	> 320	> 320	160
10	5 yrs	—	> 320	160	80	40	> 320	> 320	160
11	9 yrs	—	> 320	160	160	—	> 320	> 320	160
12	12 yrs	80	> 320	160	80	160	> 320	> 320	160

All patients were being treated with N methylglucamine antimoniate (glucantime Specia) during the course of the experiment. Only one patient was in recurrence, the others were treated for the first time

Results

There was no agglutinin activity against T.A.B detected prior to immunisation in both patients and controls. Anti-O agglutinin was absent or yielded very low titer in both patients and controls after immunisation (Tables 1 and 2) Controls had yielded good agglutinin activity against H antigen of all three organisms although the anti-TH were of higher titer than the anti-AH and still higher than the anti BH titer (Table 1) On the other

TABLE 2 *Titers of anti-O and anti H antibodies one week after the 2nd and one week after the 3rd immunizing dose of T.A B vaccine in 12 patients with Kala-azar*

		Titers of antibodies one week after the 2nd dose of T.A.B. vaccine				Titers of antibodies one week after the 3rd dose of T.A.B. vaccine			
Case N	Age	Ant TO	Anti TH	Anti AH	Anti-BH	Anti-TO	Ant TH	Anti-AH	Anti-BH
1	12 mo	—	40	—	—	—	80	—	—
2	14 mo	—	—	—	—	—	—	—	—
3	17 mo	—	—	—	—	—	160	—	—
4	18 mo	—	—	—	—	—	80	—	—
5	23 mo	—	> 320	80	80	—	> 320	60	40
6	2 yrs	—	80	—	—	—	160	40	—
7	3 yrs	40	320	—	—	—	> 320	—	—
8	3 yrs	—	> 320	80	40	—	> 320	80	80
9	4 yrs	—	—	—	—	40	160	—	—
10	5 yrs	—	40	—	—	—	80	—	—
11	9 yrs	—	—	—	—	—	—	—	—
12	12 yrs	—	—	—	—	—	160	60	40

hand, patients with Kala-azar (Table 2) yielded poor antibody activity as a whole. Anti-TH titers were particularly low one week after the 2nd immunising dose in Kala-azar patients, where in only three cases out of 12 was there a good antibody response in another three there was some antibody response and in the remaining six cases there was no antibody activity detected. However after the 3rd immunising dose most of the cases of Kala azar yielded antibody activity to anti TH although not to the same extent as controls. Anti-AH and BH antibodies were of lower titers in Kala-azar patients than in controls (Fig 1)

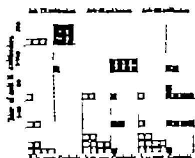


Fig. 1 Titer of anti-H antibodies one week after 2nd immunising dose of T.A.B. accine in 12 patients with Kala-azar and 12 controls.

Comment

From our data there appears to be a poor antibody production after T.A.B immunisation in patients suffering from Kala-azar anti H antibody response being particularly less than in controls after the 2nd immunising dose of T.A.B vaccine We believe that this could be significant and could well suggest an immunologic deficiency

In Kala-azar there is proliferation of reticuloendothelial and of plasma cells, blood serum proteins are altered with greatly diminished albumin and markedly increased γ globulin [10]. A number of investigators [2 4 9] believe this γ-globulin to be abnormal in nature and to be produced by the active reticulum cells.

Porges [8] described cases of myeloma in which there was a deficiency of normal γ-globulin despite the excess of an abnormal protein. In other diseases involving the lymphoreticular system [5 6] antibody production is impaired in spite of the large amounts of γ-globulin found in these diseases. It could be possible that Kala-azar protozoa interfere with normal γ globulin synthesis, both native and antibody γ-globulin, thus causing an immunologic deficiency reflected by poor antibody production, despite the large amounts of "abnormal γ globulin formed.

Another factor that may play its part could also be the hypoalbuminemia since poor antibody response has also been detected in cases of hypoalbuminemia secondary to malnutrition [1 11]

Kala-azar is a curable disease and both histological and plasma electrophoretic changes revert to normal after successful therapy We think that the immunologic deficit disappears after treatment. This could well explain the relatively better responses obtained one week after the 3rd immunising dose, as in the cases studied treatment with glucantime was started upon establishment of the diagnosis.

In conclusion, during the course of Kala-azar there is a poor antibody production which may well be due to a disturbance of protein synthesis, caused by the parasite.

therapy Clampang of the umbilical cord was accomplished in one of two ways in 10 infants it was done within 2 to 10 seconds after delivery of the trunk (*early* cord clamping) and in 22 others it was performed after cessation of the umbilical arterial pulsations which was usually within 3 to 5 minutes after the delivery (*late* cord clamping) The birth weights of the subjects ranged from *910 g to 4680 g the mean birth weight of the early-clamp infants was 3490 g and that of the late-clamp infants 3710 g

All studies were performed prior to the infant first feeding The subject was placed on a padded board over a heated table, and was carefully restrained with diaper cloths after placement of the limb electrodes for electrocardiographic monitoring A microphone was also fixed over the midprecordium to enable continuous phonocardiographic recording No medicaments, anesthetics or artificial measures were employed to keep the babies quiescent aside from the occasional use of a rubber nipple when necessary All infants were breathing room air during the entire procedure. Room temperature was maintained at 24°C to 26°C. Rectal temperature was obtained just before and after the termination of each study

Under strict surgical asepsis, the cord freshly cut allowing a stump f p- tely 2 cm to remain. One umbilical ery and the umbilical vein were then cannulated with soft polyvinyl nasogastric feeding catheters containing two side holes 6 to 7 mm apart (Sterilon Corp) A 5F catheter was always used for the artery whereas a 5F or 8F catheter was used for the vein. The proximal end of the saline-filled catheters was connected to an Elema-Schönander pressure transducer (pressure range, 0 to 300 mm Hg) and recorded by a multi channel jet writing oscillograph (Mingograf 81). Zero reference for the pressure readings was set at the anterior axillary line corresponding to 15 to 30 mm bove the midchest level. Mean pressures were obtained through electrical integration.

The arterial catheter was advanced in retrograde fashion into the aort up to a distance of 24 to 28 cm from the umbilical ring where, at this level, the recorded pressures were generally either those of the aorta upstream of the ductus arteriosus (proximal aorta) or of the pulmonary artery entered in the ductus arteriosus. With further manipulation of the catheter at the pulmonary artery site the right ventricle could sometimes be entered. The venous catheter was likewise advanced up to a distance of about 10 to 15 cm from the umbilical ring until venous pulsations were obtained. From this site which usually was the right trium, the catheter could often be advanced farther into the left atrium across the foramen ovale to a distance of about 15 to 20 cm, or sometimes into the left or right ventricle.

Cardiac fluoroscopy was not sed. The location of the catheter tip was estimated from the distance of the catheter tip from the umbilical ring, from the continuously monitored pressure curves, and from blood oxygen determinations. Each time a chamber was entered, pressures were recorded simultaneously with the electrocardiogram and phonocardiogram, and blood samples were obtained anaerobically into 2 ml heparinized syringes provided with mixing discs. Each blood sample was analyzed within 5 to 10 minutes after withdrawal for pH, pCO_2, CO content standard bicarbonat and base excess using the micro-Astrup technic [20 21]. Blood pO was measured by polarography using a Clark type electrode (E 5044, Radiometer Copenhagen) In addition, oxygen saturation was determined by reflection photometry using a Kipp-Zonen haemoreflector unit (1959 model) and the hematocrit values of several samples were checked [10].

Whenever possible the blood samples were obtained simultaneously or in rapid sequence from the venous and arterial catheters following their tentative localization at certain ideal sites. For example the pulmonary artery sample was paired with that of the right ventricle or right atrium, and the proximal as well as descending aortic samples with that of the left atrium, to detect for shunting across the ductus arteriosus.

Total blood loss from the late-clamp babies was less than 8 ml and that from the early clamp infants much less, since the determinations in some samples, particularly the repeat ones, were confined to gas tension only. In the latter instances where only pO was determined, the corresponding oxygen saturation was derived from a blood oxygen dissociation curve prepared from a large number of venous and arterial blood samples of newborn infants (14).

The pressure tracings were recorded only while the baby was quiet. The phonocardiogram and, especially the atrial or even the portal sinus pressure curves provided excellent information relative to the respiratory cycles of the baby. Thus, arterial pressures recorded during spontaneous Valsalva like maneuvers of the infant which could have been overlooked if these reference guides were not present, were discarded. Likewise, the arterial curves showing minimal respiratory excursions were preferred to those with larger respiratory fluctuations. The temporal relationship of the aortic tracing to that of the first heart sound was always checked to roughly detect whether the catheter tip presumably advanced to the proximal aortic level has not really taken an adventitious course distally to the iliac or femoral artery. In the former position, the curve immediately follows the first heart sound, whereas in the latter (or abdominal aortic) position, a short time interval elapses between the termination of the first sound and the onset of the curve. Continuous pressure recording was done each time the arterial catheter was withdrawn from the pulmonary artery to the descending aorta, or vice versa. In the 7 cases where the pulmonary artery was not entered but the right ventricle was by the venous catheter from the right atrium, simultaneous ventricular and aortic pressure curves were taken.

At the end of the procedure, the free ends of the catheterized vessels were ligated and a purse-string silk suture was placed around the umbilical stump. No complications occurred, and the babies were in good condition at the time of their discharge.

Results

The pulmonary artery was entered by the arterial catheter via the ductus arteriosus in 27 infants. Entrance into the pulmonary artery from the aorta was based on a change in the arterial pressure (to be described), significant fall in arterial oxygen saturation to 90 per cent or less, or entrance into the right ventricle with subsequent pull back to the pulmonary artery. The right ventricle was entered in 11 instances; in 3 of these the entry route was by way of the ductus arteriosus and pulmonary trunk into the outflow tract (arterial route) whereas in the 8 others it was from the right atrium (venous route). The left ventricle was also entered in 6 subjects—in one case by the arterial catheter and in the other 5 cases by the venous catheter via the left atrium. In 10 infants, the venous catheter could not be advanced into the right atrium, accordingly only portal sinus pressures and samples were obtained.

A summary of the hemodynamic findings is presented in Table 1. No attempt was made to quantitate the shunt flows due to the inability to obtain blood samples at ideally different pulmonary artery sites without the aid of cardiac fluoroscopy and to failure to sample the right ventricle in all the cases. A left-to-right shunt across the ductus arteriosus was suspected when the pulmonary artery was demonstrated to have higher oxygen saturation of more than 5% over that of the right ventricle, or greater than that of the right atrium by at least 10%. A right-to-left ductal shunt was presumed to be present if the descending aorta had an oxygen saturation of less than 95% and at the

same time, was less than that of the proximal aorta (or left heart chambers) by 4 or more % O_2 saturation. It was also suspected when the descending aorta had a comparatively lower pO_2 of more than 10 mm Hg. The latter criterion has been formulated following studies on the biologic variations of repeat proximal aortic and descending aortic pO_2 of 42 newborn infants using the present method [14]. The average pO_2 difference between the proximal aorta and descending aorta ascribable to physiologic variations, using the formula $E = \sqrt{\Sigma D^2/2(n-1)}$ where E represents the biologic variation, D = difference in pO_2, and n = number of observations, was ± 4.7 mm Hg and in only 2 out of 77 observations was the difference more than 10 mm Hg (1_ and 14).

A left-to-right ductal shunt was suspected in 5 of the 9 late-clamped infants and in ? of the 6 early-clamped subjects where this information could be obtained. In 17 instances no information relative to the existence of this shunt was available due to failure to enter the pulmonary artery in 7 and to enter the right heart chambers in the rest. A right-to-left ductal shunt was observed in 4 of the 24 infants where this information was available. In 8 other cases, the existence of this shunt could not be ascertained, due to failure to enter the ascending aorta (or left heart chambers) for comparison of its O_2 content with that of the descending aorta.

It was f lt that quant tation of shunt flows in the present study would not be too feasible. Samples obtained from the pul monary artery without fluoroscopic guid ance is always subject to the possibility of streaming effect from a funct onally patent ductus arteriosus. This may tend to exaggerate the degree of arterialization of the pulmonary arteries. The utilization of samples obtained from the right atrium (in the cases where the right ventricle is not entered) to represent mixed venous blood is likewise not too reliable since this is known to be a poor mixing chamber. We are of the opinion that dye-dilution studies utilizing the two-catheter technic would be more informative in this regard. This has been recently instituted in our laboratory using the same arterial and venous approach with promising results.

To validate comparison of the pulmonary artery pressures of the two groups of subjects at different ages, the pulmonary artery mean pressure was related to that of the corresponding aortic pressure in the form of a pulmonary aortic (P/A) ratio. The values were obtained from the continuous pulmonary—to—descending aortic withdrawal tracings. In 7 cases where the pulmonary artery was not entered, the ratio of the right ventricular systolic pressure to that of the simultaneously obtained aortic systolic pressure was used. The above values for the two groups of infants have been plotted against age in Fig. 1. During the first 9 hours, the pulmonary artery pressures of the late-clamped infants were in the systemic range and the P/A pressure ratios were about 90 %. In contrast, the early-clamped subjects manifested early and remarkable fall in pulmonary arterial pressures to as low as 70 % that of the corresponding aortic pressures by the second hour and to nearly 50 % by the fourth hour.

The drop in pulmonary artery mean pressure was accompanied by a comparatively faster decline of its diastolic than of its systolic pressure. Thus in *Fig. 2* where the P/A systolic pressure ratio was plotted

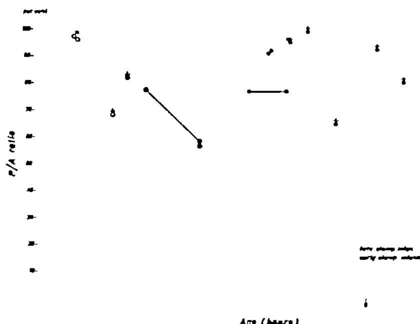


Fig. 1. Pulmonary/aortic mean pressure ratio plotted against age. Each point corresponds to one subject; those joined by line belong to the same infant. Circle with overlying + represents right ventricular/aortic systolic pressure ratio.

against the corresponding P/A mean pressure ratio, the former values were almost consistently higher than those of the latter especially in the range where the pulmonary artery mean pressures were already less than 80 % of the systemic pressures. The few points in Fig. 1 representing the RV/A systolic pressure ratio must, therefore, be interpreted in the light of this finding—their corresponding P/A mean pressure ratios must have been actually lower.

Figs. 3 and 4 are representative continuous withdrawal pressure tracings from the pulmonary artery to the descending aorta, obtained from the two groups of subjects. The early decline of the pulmo-

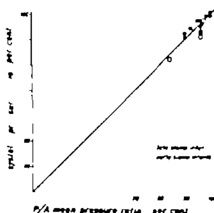


Fig. 2. P/A systolic pressure ratio plotted against P/A mean pressure ratio. See text.

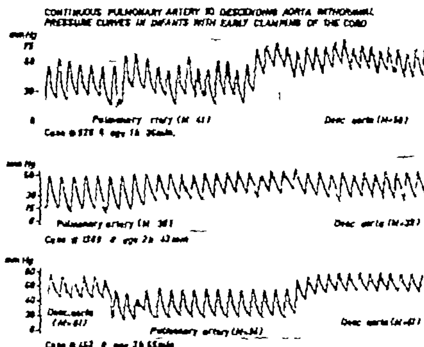


Fig. 3. Typical continuous withdrawal pressure curves from pulmonary artery to descending aorta (via ductus arteriosus) in 3 early-clamped infants.

TABLE 1 *Summary of hemodynamic data*

Subjects: Age group Type[a]	Subjects: No. cases	Subjects: A Hcrt	Intravascular pressure (mm Hg)[b]: Pulm. art. S	Pulm. art. D	Pulm. art. M	Aorta S	Aorta D	Aorta M	P/A ratio[c]	Av. blood O_2 saturation (%): R.V	R.V	P.A.	L.A. & Prox. A.	D.A.	Ductal shunts[d]: L–R P	L–R A	L–R U	R–L P	R–L A	R–L U
3 hours																				
LC	4	37	75	47	59.5	77	48	61	93	91	—	86	96	96	—	1	3	1	1	2
EC	8	46	57	27	39.5	67	37	48	80%	83	83	84	97	93	2	4	2	1	6	1
3–7 hours																				
LC	13	39	63	26	4 5	67	42	34.8	91	81	91	84	96	94	3	2	8		6	3
EC	1	40	54	21	34	77	31	61	58	—	—	83	—	94	—	—	1	—	—	1
7 hours																				
LC	5	59	50	30	38.3	65	43	53	77%	84	85	84	97	96	—	—	5	—	4	1
EC	1	38	48	13	26.3	68	44	52	51%	—	—	82	98	95	—	—	1	—	1	—

[a] LC = late-clamp group; EC = early-clamp group.
[b] S = systolic; D = diastolic; M = mean.
[c] Pulmonary/aortic mean pressure ratio; also includes right ventricular/aortic systolic pressure ratio in those where the pulmonary artery was not entered.
[d] L–R = left-to-right; R–L = right-to-left; P = present; A = absent; U = undetermined. See text.

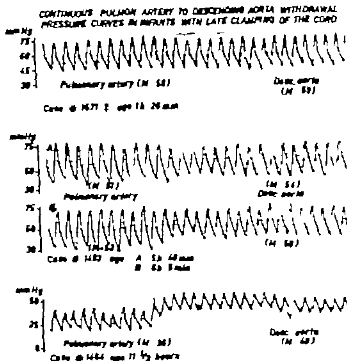


Fig. 4. Typical continuous withdrawal pressure curves from pulmonary artery to descending aort (la ductus arteriosus) in 3 late-clamped infants.

nary artery pressures in the early-clam ped infants is again evident. In addition, the occurrence of a diastolic pressure dif ference even in the presence of nearly equivalent systolic pressures is shown. In the cases, however where the pulmonary mean pressure has already dropped to about 3/4 that of the aortic, a significant pressure difference was already present during both systole and diastole (Figs. 3 and 4)

Discussion

A number of investigators have studied the pulmonary artery pressures of normal newborn infants, and in t least 4 the investigations were mostly confined to the immediat newborn period [1 8, 18 19]. Those have been summarized by Emmanouilides *et al.* [8] in a scattergram correla ting the pulmonary arterial mean pressure with ge of 85 normal term infants (including 51 of their own cases) less than one hour to 3 days of age These previous investigations have conclusively demon strated rapid decline of the pulmonary arterial pressure sometime during the first day and a gradual fall thereafter to approximately adult levels in the next few days. During the first 60 minutes of life Saling [19] found the pulmonary artery pressures to be equivalent to or even higher than that of the aorta in at least half of his subjects. Between 1 and 10 hours, the pulmonary arterial pressures tend to remain high and, not uncommonly close

to systemic levels, thereafter a rapid fall in pressure occurs during the rest of the first day [8]

A common observation in these previous studies has been the wide variability of the pulmonary arterial pressure levels among different subjects during the early hours of life. In none of these investigations was the degree of placental transfusion, in terms of early or late clamping of the cord, correlated with these observed variations. From our present findings, it is conceivable that those cases with *significantly* low pulmonary pressures (relative to the aortic pressures) may have been the recipients of smaller placental transfusion than those with high pulmonary pressures.

A pertinent question is what accounts for the striking difference between the pulmonary arterial pressures of the two groups of subjects in the very early newborn period. The difference is real since biologic variation among individual subjects (also reflected in the systemic arterial pressures) has been corrected for by utilizing the P/A pressure ratios for comparison. The etiology is presently not known but it must be intimately related to the total blood volume of the early clamped and late-clamped subjects which has been shown to be approximately 25 to 30% higher in the latter infants during the first half hour evidently due to greater placental transfusion that has transpired during the delivery [13–23]. In support of this assumption were the elegant studies of Wallgren and co-workers [24] dealing with the hemodynamic effects of acutely induced hypo- and hypervolemia on 15 erythroblastotic infants prior to an exchange transfusion. These workers have observed that step-wise withdrawal of approximately 25% of the infant's estimated total blood volume resulted in significant reduction of the heart size, right heart filling pressures, and pulmonary as well as systemic pressures; conversely transfusion of approximately 25% in excess of the original blood volume resulted in an increase of the above parameters. It should be pointed out, however, that these studies consisted of, understandably short-term observations upon relatively older newborn infants of which only – were less than 24 hours of age and as such do not necessarily reflect the hemodynamic events transpiring in newborn infants subjected to maximal or minimal placental transfusion.

How can the size of the total blood volume be related to the pulmonary arterial pressure levels in the early newborn period? Large pulmonary blood flow perhaps due to higher cardiac outputs may be implicated in the late-clamped infants. However cardiac output has no direct relationship with total blood volume. Experimentally induced hypervolemia in man [22–25] and in dogs [9] has been observed to be accompanied by an increase of right heart filling pressure but not by significant changes in cardiac output. Likewise, moderate hypovolemia in dogs has been observed not to alter the cardiac output [3]. In congestive heart failure where total blood volume is increased, there is often some reduction of cardiac output. The larger heart size observed roentgenologically in newborn infants allowed maximal placental transfusion [4] could very well be due to greater residual blood content in the heart than to increased stroke output.

There is convincing evidence for the existence of active vasoconstriction of the small pulmonary arteries in newborn animals [_, 5, 6 16 17] as well as in newborn humans [11, 12], and the resulting high pulmonary vascular resistance must obviously be the chief factor accounting for the pulmonary hypertension during the immediate newborn period. The vasoconstriction has been assumed to be due to low arterial oxygen tension [6 11 16] which can be released by elevating the arterial oxygen content or tension [6]. We have not observed any significant difference in the arterial oxygen tension or saturation, as well as in the acid base state between the 2 groups of subjects;[1] consequently the disparity in the pulmonary artery pressures cannot be related to the blood gas content of these subjects. We suspect that the higher pulmonary arterial pressures in the late-clamped infants during the early hours of life is due to comparatively greater pulmonary vasoconstriction (higher pulmonary vascular resistance), secondary to greater filling or distention of the pulmonary capillary venous bed, in the same way that it probably occurs in the other vascular compartments of the body It is conceivable that the vasoconstriction may involve not only the small muscular arteries but perhaps also the pulmonary veins which has been shown to be capable of a similar vasomotor activity [15] In the early clamped babies, where the blood volume is small, these pulmonary vascular responses are presumably less, and this may account for the lower pulmonary arterial pressures at this early age

The above hypothesis has several theoretical implications. It serves to explain the pulmonary vasoconstriction in the immediate newborn period independently of the arterial pO_2. It may also account for the "secondary" pulmonary hypertension observed in severe pulmonary congestive states such as in cor triatriatum, congenital mitral stenosis or left heart failure.

Summary

Hemodynamic studies were conducted on 32 normal, full term newborn infants, age 29 minutes to 11 hours, to determine the effect of placental transfusion upon the pulmonary circulatory adjustments after birth. In 10 infants, clamping of the cord was accomplished within 2 to 10 seconds after the delivery (*early* clamping), and in the other 22 infants it was done after cessation of the umbilical arterial pulsations (*late* clamping)

For comparative purposes, the pulmonary arterial pressures were related to the corresponding aortic pressure in the form of a P/A mean pressure ratio, and these were correlated with age in the groups of subjects The observed pulmonary arterial pressures in the groups differed significantly During the first 9 hours, the pulmonary pressures of the late-clamped infants were approximately 00 that of the aorta. In contrast, those of the early-clamped infants were as lo as 70% of the aortic pressures by [illegible] second hour and nearly 60% by [illegible] fourth hour

A hypothesis is presented that [illegible] er pulmonary arterial pre[illegible]

[1] The acid-base data of the present series ha been incorporated with much larger series and ill be reported.

late-clamped infants is due to greater pulmonary vasoconstriction resulting from greater pulmonary capillary—venous filling—a consequence of the large blood volume following maximal placental transfusion.

References

1. Adams, F H. and Lind J Physiologic studies on the cardiovascular status of normal newborn infants (with reference to the ductus arteriosus). *Pediatrics, 19* 431 1957
2. Adams, F H., Hirvonen L., Lind J and Peltonen T Physiologic studies on the cardiovascular status of newborn pigs effect of adrenaline noradrenaline acetylcholine and serotonin. *Etudes néo-natales, 7* 53, 1958.
3. Barrett, H. O. Blood volume and cardiovascular adjustment. *Amer Heart J 21* 422, 1941
4. Burnard, E. D and James, L. S.: Radiographic heart size in apparently healthy newborn infants clinical and biochemical correlations. *Pediatrics, 27* 726, 1961
5. Cook, C. D Drinker, P Jacobson H., Levison H., and Strang L.: Control of pulmonary blood flow in foetal and newly born lamb *J Physiol, 169* 10, 1963.
6. Dawes, G S. and Mott J C.: The vascular tone of the foetal lung. *J Physiol, 164* 10, 1962.
7. DeMarsh, Q B., Windle, W F and Alt H. L. Blood volume of newborn infants in relation to early and late clamping of the umbilical cord. *Am J Dis Child, 63* 1123, 1942
8. Emmanouilides, G C Moss, A. J Duffie, E. R., Jr. and Adams, F H.: Pulmonary arterial pressure changes in human newborn infants from birth to 3 days of age *J Pediat, 65* 327 1964.
9. Fewell, V O Bloom, W L. and Ward J A. Hemodynamic effects of hypervolemia with and without anemia. *Circ Res 6* 163 1958.
10. Guest, G M. and Siler, V E.: Centrifuge method for determination of volume of cells in blood. *J Lab Clin Med, 19* 757 1934.
11. James, L. S. and Rowe, R. D The pattern of response of pulmonary and systemic arterial pressure in new born and older infants to short periods of hypoxia. *J Pediat 51* 511, 1957
12. Moss, A. J., Emmanouilides, G C Adams, F H. and Chuang, K. Response of the ductus arteriosus and systemic arterial pressure to changes in oxygen environment in newborn infants. *Pediatrics, 33* 937 1964.
13. Oh, W Blankenship W and Lind J Further blood volume studies on newborn infants during the first 2 hours of life in relation to placental transfusion. To be published.
14. Oh W Arcilla, R. A. and Lind J In vivo blood oxygen dissociation curve in newborn infants. *Biol Neonat,* In press.
15. Parker, B. M., Smith, B. W and Friedenberg, M. J Serotonin-induced pulmonary venous spasm demonstrated by selective pulmonary phlebography *Amer Heart J 69* 521 1965.
16. Reeves, J T and Leathers, J E.: Pulmonary vasoconstriction in the newborn calf. *Fed Proc, 22* 453, 1963 (abst.).
17. Rudolph, A. M., Auld P A., Golinko, R. J and Paul, M. H.: Pulmonary vascular adjustments in the neonatal period. *Pediatrics, 28* 28, 1961
18. Rudolph, A. M., Drorbaugh, J E., Auld P A. M., Rudolph, A. J Nadas, A. S., Smith C. A. and Hubbell, J P Studies on the circulation in the neonatal period: the circulation in the respiratory distress syndrome. *Pediatrics, 27* 551, 1961
19. Saling E.: Neue Untersuchungsergebnisse uber den Kreislauf des Kindes unmittelbar nach der Geburt. *Arch Gynäk, 194* 287 1960
20. Siggaard Andersen O Engel, K., Jorgensen K. and Astrup P A micro-method for determination of pH, carbon dioxide tension, base excess and standard bicarbonate in capillary blood. *Scand J Clin Lab Invest, 12* 177 1960
21. Siggaard-Andersen O The pH log pCO blood acid-base nomogram revised. *Scand J Clin Lab Invest, 14* 598, 1962.
22. Thomasson B A study of right heart performance in man with special reference to the effect of experimentally induced acute hypervolemia. *Scand J Clin Lab Invest, 11* (Suppl. 40), 1959.
23. Usher, R., Shephard M. and Lind, J The blood volume of the newborn infant and placental transfusion. *Acta Paediat Scand, 52* 497 1963.
24. Wallgren G Barr, M. and Rudhe, U Hemodynamic studies of induced acute hypo- and hypervolemia in the newborn infant. *Acta Paediat Scand 53*, 1 1964.

25. WARREN, J. V., BRANNON, E. S., WEENS, H. S. and STEAD, E. A., JR.: Effect of increasing the blood volume and right atrial pressure on the circulation of normal subjects by intravenous infusions. Amer J Med, 4 193, 1948.

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Incidence in infants and Mortality from Congenital Malformations of the Circulatory System

by K. F. KERREBIJN

Congenital malformations of the circulatory system account for a great proportion of all congenital malformations. Mortality in infants from congenital malformations of the circulatory system is high [2, 4, 7 9 11]. Research into the incidence of these conditions will give the most reliable results if conducted during the first year of life. A disadvantage of examination at such an early age is however that certain malformations may be less easily recognised at this than at a later stage [3 5 10 11]

Investigation by means of screening in the form of history taking and physical examination, performed by a physician experienced in the field of cardiological diagnosis, is feasible and has a good sensitivity [8]. On account of the lack of specificity of the screening, overdiagnosis will occur. This creates no problem since complete cardiological examination will be necessary in all those cases where signs of a possible abnormality are found.

The present investigator has looked into the incidence of congenital malformations of the circulatory system and has estimated the number of children born alive annually in the Netherlands with such abnormalities. He also has analysed and reviewed the mortality from congenital malformations of the circulatory system as an underlying cause of death in the Netherlands over the years 1951–1962.

Material and Methods

The incidence of congenital malformations of the circulatory system has been investigated amongst all live births in 1958 among the inhabitants of the municipality of Leiden, the Netherlands (hereafter referred to as the research group)

The children from the research group who were examined had either been referred to the present investigator by general practitioners, pediatricians, cardiologists and infant welfare-clinic physicians in Leiden or had been screened by means of history taking and physical examination by the author during the poliomyelitis immunisation organised by the Public Health Services in 1959

Extensive cardiological investigation (X ray electrocardiogram, phonocardiogram and if indicated cardiac catheterisation and/or angiocardiography) were carried out on all the children referred to the investigator and on all those in whom a malformation of the circulatory system seemed possible as adjudged at the time of screening

A questionnaire concerning points relevant to the existence of a malformation of the circulatory system was sent to the parents of those children from the res-

TABLE 1 *Types of conge ital malformations found*

Diagnosis	Number
Patent ductus arteriosus (Botalli)	1
Ventricular septal defect	6
Auricular septal defect	1
Pulmonary stenosis	1
Ventricular septal defect combined with pulmonary stenosis	1
Ventricular septal defect combined with auricular septal defect and pulmonary stenosis	1
Truncus arteriosus	1
Atresia of the ostium aortae with rudimentary left ventricle	1
N diagnosis (1 malformation with cyanosis)	2

arch group who had not been examined by the investigator The xamined and not-exa mined children were compared demographically (maternal age, paternal age, number of children previously born by the mother parental profession).

Mortality figures were collected from data available from the Central Bureau for Statistics.

Results

Investigation of incidence

The research group consisted of 1817 children. 1408 (77.5%) were examined at the age of 2–15 months, in over 90% by means of screening 255 (14 0%) were not examined but their families answered the questionnaire (In one a congenital mal formation of the circulatory system was present.) 133 (7..) were not examined and their parents did not answer the questionnaire (in 40 because they had moved to other towns) 21 (1 1%) had died and had not been examined by the investigator during their lif It was possible to trace the causes f death on the death certificate in 17 cases.

In 15 (8‰ of the research group) a congenital malformation of the circulatory system was found (T ble 1) In all cases diagnosis was established by ca theterisation and/or angiocardiography and/or autopsy

Investigation of mortality

The mortality from congenital malfor mations of the circulatory system occurs for 80% during the first year of life (Table) The number of infants dying from congenital malformations of the circulatory system is 2 per 1000 live births, that is to say at the moment about 500 annually in the Netherlands The proportion of total infant mortality attributable to these malformations has increased from 7 6% in 1951 to 13 7% in 1962. One third of all these infants die in the first week of life half within the first month.

The mortality in the 1–14-year age-group from congenital malformations of the circulatory system is increasing However if expressed as a percentage of total live births in the relevant birth years, no change in incidence has occurred since 1954 (Table 2)

Discussion

The children examined can be considered representative of all the children from the research group as assessed by demographic comparisons. The results of the investigation can also be considered representative for the Netherlands as a whole since there is no important difference demographically between the population of Leiden and the average Dutch population and since there is a close similarity between the mortality figures from congenital malformations of the

TABLE 2 *Deaths in the 0–14-year age-group from congenital malformations of the circulatory system 1951–1962*

Age		Period			
		1951–1953	1954–1956	1957 1959	1960–1962
<1	Total number	1228	1237	1350	1443
	Annual average	409	412	433	481
	As a % of total deaths from congenital malformations of the circulatory system in the 0–14-year age group	83	83	80	81
	Per 1000 live births	1.8	1.8	1.9	1.9
1–4	Total number	171	159	208	190
	As a % of total deaths from congenital malformations of the circulatory system in the 0–14-year age group	11	10	12	10
	Per 100 000 live births	8	8	7	9[a]
5–14	Total number	88	104	141	161
	As a % of total deaths from congenital malformations of the circulatory system in the 0–14-year age group	6	7	8	9
	Per 100,000 live births	2	2	2	2[a]
	Grand total	1487	1499	1708	1793

The years 1960–1962 taken separately show the same picture.

circulatory system (which can be considered to reflect the incidence of these malformations) for the municipality of   and those for the Netherlands as a whole [6].

Probably mistakes have been made in the calculation of the reported incidence of congenital malformations of the circulatory system (1) It is possible that some malformations are not discovered. (2) In calculating the incidence of congenital malformations of the circulatory system the total size of the research group (1817) is in ratio to the number in whom a malformation has been detected (15) In establishing the incidence it is assumed that none of the 387 children who were not examined had a congenital malformation of the circulatory system This is of course not certain. Thus the possibility must be taken into consideration that the established incidence of congenital malformations of the circulatory system in the research group is too low The group of children examined comprised both those who were screened by the investigator during inoculation and those who were referred to him by other doctors. Children in this second category constitute a major proportion of the total number of malformations discovered (8 out of the 15) Calculation of the incidence with respect only to the children examined would thus have given too high a figure. (3) Particulars are not known of all deaths and a post-mortem was not always carried out It is possible that more often than is known a congenital malformation of the circulatory system was present.

It must be concluded that the incidence

calculated is if anything too low rather than too high.

Assuming that 8‰ of the live births have a congenital malformation of the circulatory system means, that at the moment in the Netherlands (population about 12 millions, live births annually about 250,000) about 2000 children would be born alive with such a malformation. It can be calculated from mortality figures (Table 2) that the number of children with a congenital malformation of the circulatory system who reach schoolage is about 1400, that is to say about 6‰ of the total living four year-olds in the Netherlands. This is approximately in accordance with the incidence found here and in different parts of the world.

It may be assumed that the spread of the found incidence of congenital malformations of the circulatory system in the live births in 1958 among the inhabitants of the municipality of Leiden (8‰), which can be assessed employing statistical calculations with reliability of 0.95 as lying between 4–12‰, will in actual fact be much less. There is no reason to assume that the incidence of congenital malformations of the circulatory system in Leiden was accidentally higher in 1958 than in other years. Therefore it is highly improbable that, if such incidence were to be assessed over a longer period of time it would prove to be lower than the incidence calculated in the present study It is possible that it is higher If it should be 10‰ of live births, that would mean that the number of children with a congenital malformation of the circulatory system who reach school-age could be about 8 per 1000. Such a high figure has, however never been reported in school health examinations.

The incidence found in this investigation can be compared with the result of the investigations by Richards *et al* [11] who found an incidence of congenital malformations of the circulatory system of 7.6‰ and of Carlgren [1] who found an incidence of 6.4‰.

The mortality figures for congenital malformations of the circulatory system are derived from death certificates issued by the doctor concerned, who is often a specialist.

Mistakes are doubtless made in the diagnosis of the cause of death. The constancy of the mortality figures since 1951 provides however some basis for the supposition that these mistakes have no noticeable influence on the pattern of the mortality figures. This is also the case regards the possibility that greater knowledge and better diagnosis of congenital malformations of the circulatory system has increased the notification rate so that an actual decrease in the mortality figures might have been camouflaged.

While infant mortality in the Netherlands as a whole is decreasing steadily it has not yet been possible to register a decrease in infant mortality from congenital malformations of the circulatory system.

Because this mortality ensues in 80% of the cases in infancy any change will mainly be the reflection of an alteration of mortality in the first year of life. A modification in the slight total number of deaths will however have to be fairly large if it is to be reflected in the mortality figures. A considerable proportion of the mortality is attributable to malformations which can now be treated in a way that is of major benefit to the patient. If all the infants concerned are treated sufficiently early there will probably be a decrease in the infant mortality from

congenital malformations of the circulatory system. Therefore it is urgent that these malformations be recognised in time

Summary

This study comprises an investigation into the incidence of congenital malformations of the circulatory system in all live births in 1958 among the inhabitants of the municipality of Leiden and an analysis of the figures concerning congenital malformations of the circulatory system as an underlying cause of death in the Netherlands in the years 1951–1962

In 15 of 1817 children (8‰) a congenital malformation of the circulatory system was found. A discussion is included of possible sources of error in the calculation of the incidence reported.

The results can be considered as representative for the Netherlands as a whole. This means that the number of infants born alive in this country with a congenital malformation of the circulatory system will currently be about 2000 annually

Up to the present there has not been a decline in mortality figures for congenital malformations of the circulatory system in the Netherlands. Mortality ensues in 80% of the cases in infancy. The number of infants dying from congenital malformations of the circulatory system is at the moment about 500 annually. One third die in the first week of life half within the first month.

References

1 Carlgren L. E.: The incidence of congenital heart disease in children born in Gothenburg 1941 1950. *Brit Heart J* *21* 40, 1959
2. Centraal Bureau voor de Statistiek: *Mortaliteit door aangeboren misvormingen 1950–1961* de Haan N V Zeist 1963.
3. Chapman C and Fraser, R.: Clinical and hemodynamic features of uncomplicated interatrial defects in adults. *Amer Heart J* *46* 362, 1953.
4 Gardiner, J H. and Keith, J D Prevalence of heart disease in Toronto children. *Pediatrics*, *7* 713, 1951
5 Keith, J D and Forster, C C Auricular septal defects in children. *J Pediat*, *38* 172, 1951
6. Kerrebijn K. F *Pediatric cardiology in the Netherlands* Thesis, Leiden 1964.
7 MacMahon B. McKeown T and Record R. G The incidence and life expectation of children with congenital heart disease. *Brit Heart J* *15* 121, 1953.
8. Morton W Hoffmann M. S., Clemmer, R. L. and Arnold R. C.: Heart disease screening in elementary schoolchildren. *JAMA* *169* 1163, 1959.
9. Mustacchi, P Sherins, R. and Miller, M J Congenital malformations of the heart and the great vessels. *JAMA*, *183* 241 1963.
10 Nadas, A. *Pediatric Cardiology* Saunders, Philadelphia 1957 1st edition.
11 Richards, M. R., Merritt, K. K., Samuels, M. H and Langmann A. G Congenital malformations of the cardiovascular system in a series of 6053 infants. *Pediatrics* *15* 12, 1955.

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CASE REPORT

Generalized Histiocytic Reticulo-Endotheliosis and Leuco-Encephalopathy in Childhood

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A report will be given on a child who clinically showed signs of grave lesions of the central nervous system. Radiological examination revealed multiple osteolytic lesions. Biopsy examination of such a lesion showed an eosinophilic granuloma. On histological examination at autopsy a generalized histiocytic reticulo-endotheliosis and a multifocal leuco-encephalopathy were found.

Case Report

This male infant was the fourth child of not related, healthy parents. His four siblings were healthy. No diseases of the nervous system are known in his family. He was born at term. Pregnancy, labor and delivery were normal. Birth weight 3300 g. Development seemed to be normal. He walked at 11 months and talked well at two years of age. At 15 months he had a febrile disease with stiffness of the neck but no other neurological signs. The spinal fluid contained 14 polymorphonuclear cells and 12 lymphocytes/mm³. Antibiotics were given and in two days he recovered completely.

At *three years of age* his gait became wide and uncertain. His feet were turned inwards. Within a couple of weeks he could not walk without support. No pareses were noticed and all reflexes were normal. Nystagmus. He also became whining and irritable. Hemoglobin was 12 g%, white cells 7800 with normal differential count. Negative serological reactions for syphilis and toxoplasmosis. Spinal fluid normal. X-ray of the skull and skeleton showed localized osteolytic lesions in the left temporal, parietal and occipital bones (Fig. 1). Similar lesions also occurred in the pelvis, in the left scapula and in a rib. Electro- and pneumoencephalography showed normal conditions. Biopsy from the lesion in the occipital bone revealed the characteristic picture of eosinophilic granuloma. A series of X-ray treatments were given and within a year the lesions had become almost completely sclerotic or had disappeared. His gait made some improvement but still he could not stand or walk without support. After an influenza-like febrile illness his disease progressed and a few short episodes of clonic fits in his arms were seen.

Examination at *five years of age*: His somatic condition revealed nothing abnormal. The liver and spleen were not enlarged. No lymph nodes could be palpated. There was no exophthalmus, polydipsia or polyuria. He was mostly rather alert, talked and was well orientated but cooperated badly. Spastic tetraparesis, ataxic gait as before and drop-foot on both sides were found. He had a slight intention tremor and poor control of head movements. Salivation was increased.

Ophthalmological examination revealed a coarse horizontal nystagmus, otherwise nothing abnormal was found. Cholesterol in blood: 245 mg%. Spinal fluid: 21 mg% total protein, no cells. A metachromatic substance was found in the urine.



Fig. 1 X ray picture of the skull showing osteolytic lesions of the occipital and left parietal bones.

His condition slightly improved. H could eat, play and mov with the help of a chair At 6½ years of ge he caught an acute respiratory disease, developed respiratory lure and died.

Histological examination of biopsy

The biopsy specimen taken from the occi pital bone t 3 years of ge was examined by Dr Fredrik Wahlgren (Södersjukhuset) and disclosed pieces of a highly cellular tissue composed mainly f reticular cells, eosinophilic leucocytes and some erythrocytes and lymphocyte-lik mononuclear cells. No xanthomatous or giant cells were found (Fig. 2).

Post mortem findings

Examination of the nervous system

The right cerebral hemisphere was taken for chemical analysis (it is hoped that the results will be published later on) The rest of the brain was fixed in 10% formol. Representative blocks were removed from the frontal parietal and occipital lobes, hypothalamic and hippocampal regions, basal ganglia and cerebellum. From these blocks paraffin sections of 10 μ were cut. They were examined histologically using the following staining methods: Luxol fast blue and Spielmeyer method for myelin, Amidoblack B, Holzer method for astrocytes and Palmgren s silver technique for axons. Cresyl violet acetic acid (v Hirsch & Peiffer) and F yrter' method for metachromasia, Schnabel s modification of Feigin s method for cholesterol and cholesterol esters [17], scarlet red and PAS were pplied to frozen sections.

Macroscopic findings. The left hemisphere sh wed no evident signs of atrophy or sclerotic changes. A marked general atrophy of the cerebellum with a sclerotic white matter was seen. The meninges and the large vessels at the base were normal.

Microscopic find ngs The cytoarchitecture of the cortex was well preserved. N focal changes were seen. The Nisl substance of the nerve cells stained w ll and no lipid deposits were found. A small patch of fibrous gliosis was seen near the end-plate of the Ammon horn, but there was no reduction of the nerve cells of the pyramidal band.

Throughout the white matter small, somewhat diffusely outlined plaques of demyelination were scattered (Fig. 3) The axons passing through these plaques were moderately degenerated showing some fragmentation and swelling. In the demyelinated areas hypertrophy and hyperplasia of the fibrous astrocytes occurred. Frequent binucleated astrocytes were also seen. Between those cells oligodendroglial nuclei and some small strands of mesenchymal cells were present (Fig. 4) No inflammatory cells, but a few macrophages containing a scarlet red positive substance were seen round the pparently normal vessels. In addition to these plaques, areas of a slight diffuse demyelination and gliosis were found in the more central parts such as in the centrum

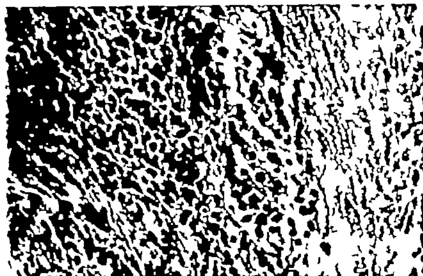


Fig. 2. Biopsy specimen from the osteolytic lesion of the occipital bone showing the granular arrangement of highly cellular tissue composed mainly of reticular cells, eosinophilic leucocytes and lymphocyte-like mononuclear cells. Giemsa. ×480.

semovale and the internal capsule No metachromatic material was detected. No cholesterol or cholesterol esters occurred in the demyelinated areas. A slight gliosis was seen in the pallidum. Scattered astrocytes with swollen, pale cytoplasm and binucleated astrocytes were found in the putamen. No cellular infiltration of the hypothalamic region was noticed.

The most marked changes were seen in the cerebellum. Abundant confluent patches of demyelination, of the same type as those of the cerebral cortex, were found (Fig 5) There was an extensive proliferation of the Bergmann glial cells in the molecular layer The Purkinje cells were markedly reduced in number Frequent degenerative axonal swellings, torpedoes were seen. There was a reduction and loosening of the granular layer in certain areas (Fig. 6). An extensive gliosis of the dentate nucleus was seen. The brainstem and spinal cord were unfortunately not available for study

Examination of the viscera

Nodular lesions of pale granular appearance were scattered throughout the lungs (Fig 7). Microscopically they consisted of large pale mononuclear histiocytes with swollen cytoplasm containing scarlet red positive droplets, which took no stain for



Fig 3. Cerebral white matter showing plaque of demyelination leaving a subcortical band of white matter intact Spielmeyer



Fig. 4. Lesion from the cerebral white matter showing demyelination, oligodendroglial nuclei and binucleated astrocytes. Luxol fast blue. × 480

cholesterol or cholesterol esters. Multinucleated giant cells were found and lymphocytes, plasma cells and neutrophile leucocytes were present (Fig 8)

In preparations from the *liver spleen* and *thymus* histiocytes with large pale sharply outlined nuclei were diffusely scattered Fatty degeneration of the liver cells around the central veins was observed.

Sections from the *pancreas adrenal, kidney* and *myocardium* appeared normal.

Discussion

Wallgren [18] suggested in 1940 that the Letterer–Siwe disease and the Hand–Schüller–Christian disease were variants of the same disorder Later it has been suggested that eosinophilic granuloma is also a manifestation of the same disease and it is now generally agreed that these three diseases represent clinical variants of a single fundamental disorder of the reticulo-endothelial system [3 10]. Histiocytic reticulo-endotheliosis has been proposed as a convenient generic term for this disorder [12] The finding of eosinophilic granuloma of the bone, which healed



Fig. 5. Cerebellum showing confluent patches of demyelination. Spielmeyer



Fig. 6. Degenerative axonal swellings, torpedoes of Purkinje cells in the granular layer of the cerebellum. Palmgren. ×90.

after X ray treatment, and the autopsy findings of nodules of foam cells in the lungs and proliferation of histiocytes in the liver spleen and thymus in the same individual, as in the present case, agrees well with the hypothesis of a nosological entity

The clinical picture offered diagnostic difficulties. As a metachromatic substance was found in the urine it was clinically considered most likely that the child suffered from metachromatic leucodystrophy and that the finding of eosinophilic granuloma was merely a coincidence The clinical finding of ataxia corresponds to the finding of grave lesions of the cerebellum, including patchy demyelination, severe and widely spread cortical degeneration and gliosis of the dentate nucleus Further correlation between neurological and pathological findings would be meaningless since only a part of the central nervous system was available for examination.

The patchy demyelination observed is, however unlike the degeneration of the white matter usually encountered in the various forms of leucodystrophy In these



Fig 7 Section through lung showing scattered granulomata with some tendency to peribronchial grouping.



Fig 8. Granulomata from the lung showing swollen, lipid-laden histiocytes and inflammatory cell infiltration. HTX-Eosin. 40

cases, which often are of familial occurrence the demyelination is more likely to be diffuse than focal. In metachromatic leucodystrophy contrary to the present case the oligodendroglial nuclei tend to be reduced in number and a metachromatic substance is detected in the cerebral white matter [15, 18]. No definite diagnostic conclusions can be drawn from the clinical finding of a metachromatic substance in the urine, since it has been found in mental defectives of all ages [11] and in normal individuals in early infancy [8].

There is no morphological resemblance between the cerebral changes in the present case and those described in cases of post-infectious disseminated encephalomyelitis

A similar multifocal leuco-encephalopathy has been described complicating cases of Hodgkin's disease, sarcoidosis, chronic lymphatic leukemia and tuberculosis [20]. The histological picture in these cases, with oligodendrocytes altered to cells with a large round densely basophilic nucleus, differed, however from that in the present case

Although the fundamental pathological changes resemble those of disseminated sclerosis (i.e. the focal demyelination with relative sparing of the axons, hypertrophy and hyperplasia of the often binucleated astrocytes and macrophages laden with sudanophilic degeneration products) they differ from the latters in being diffusely demarcated and confined to the white matter [7] The age incidence differs too although some cases considered as disseminated sclerosis have been described in childhood [ ]

Intracranial hypertension and epilepsy spastic paraplegia and cerebellar ataxia are the neurological symptoms described in Hand–Schüller–Christian disease [1]. Involvement of the nervous system in this disease has usually been described as comprising local extension of granulomata in the dura mater and the region of the sella turcica Primary scattered granulomata like those seen in the other organs have also been found in mainly involving the white matter [3 6 13 14].

However in a few cases as in the present one the cerebral lesions are different,

consisting of widely spread focal demyelination [4 9 10] and cerebellar degeneration [9 10] without any signs of granulomata It seems possible that in these cases the cerebral changes are precipitated by some myelinolytic or neuroallergenic agent released from the organs affected by the reticuloendotheliosis.

Summary

A report is given of a 6-year-old boy who at 3 years of age suddenly developed signs of ataxia Multiple eosinophilic granulomata of the bones were found, which healed on X ray treatment. Later a spastic tetraparesis and some epileptic fits complicated the picture At autopsy a multifocal demyelinating leuco-encephalopathy histiocytic granulomata of the lungs and a diffuse histiocytic proliferation of the liver spleen and thymus were found. Since a few cases of a similar demyelinating leuco-encephalopathy have been described in combination with Hand Schüller–Christian disease a clinical variant of histiocytic reticulo-endotheliosis, this combination must be more than a coincidence and has to be taken into consideration when a patient with histiocytic reticulo-endotheliosis develops cerebral symptoms.

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References

1. VAN BOGAERT L.: Le cadre des xanthomatoses et leurs différents types. In *Maladies nerveuses génétiques d'ordre métabolique*. S. A. Vaillant-Carmanne, Liège, 1962.
2. VAN BOGAERT L. and MATTHYS, E La sclérose en plaques chez l'enfant. *Acta Neurol Belg 6* 615, 1949.
3. CHIARI, H.: Über Veränderungen im Zentralnervensystem bei generalisierter Xanthomatose vom Typus Schüller–Christian. *Virchow Arch Path Anat, 288* 526, 1933.
4. DAVISON C.: Xanthomatosis and the central nervous system. *Arch Neurol Psych, 30* 75, 1933.
5. DENNIS, J W and ROSAHN, P D The primary reticular granulomas *Amer J Path, 27 (4)*: 627 1951
6. FEIGIN L: Xanthomatosis of the nervous system. *J Neuropath Exp Neurol 15* 400, 1956.
7 GREENFIELD, J G and NORMAN, R. M Demyelinated sclerosis. In *Greenfield Neuropathology* p 453, E. Arnold Ltd. London, 1963, 2nd edition.
8. HAGBERG, B SOURANDER, P and SVENNERHOLM, L. Clinical laboratory diagnosis of metachromatic leukodystrophy *Cereb Palsy Bull 3* 438, 1961
9. HALLERVORDEN J Gehirnbefunde bei Christian–Schüllerscher Krankheit und allgemeiner Cholesterinosen. *Z Ges Neurol Psych, 161* 334, 1938.
10. HEINE, J Beitrag zur Schüller–Christianschen Krankheit. *Beitr Path Anat, 94* 412, 1934/35.
11 HELLSING M. H BÖRJESSON M. and HELLSTRÖM, B The value of urinary sediment examination as screening method in suspected cases of metachromatic leucodystrophy *Acta Paediat Scand 51* 49, 1962.
12. LIGHTWOOD R., BODIAN M., SUTCLIFFE, J MACKAR, G H. et al.: Discussion: Eosinophilic granuloma, Letterer–Siwe disease, Hand–Schüller–Christian disease *Proc Roy Soc Med, 46* 711 1953.
13. MARKKOTT W.: Das Gehirn bei der Lipoidgranulomatose. *Beitr Path Anat, 110* 544, 1949.
14. MÜLLER, D Die intrazerebrale Form der Lipoidgranulomatose *Fortschr Neurol Psychiat, 31* 225, 1963.

15. PFEIFFER, J Differentiation of various types of leukodystrophies. *World Neurol*, *3* 580, 1962.
16. POSER, CH. M.: Leukodystrophy and the concept of dysmyelination. *Arch Neurol* (*Chicago*), *4* 323, 1961
17 SCHNABEL, R.: Eine topochemische Methode zur Differenzierung des freien und veresterten Cholesterins. *Acta Histochem*, *18* 101 1964
18. WALLGREN A.: Systemic reticuloendothelial granuloma. *Amer J Dis Child* *60* 471 1940.
19. WOLMAN M Eosinophilic xanthomatous granuloma and its relation to the Hand-Schuller-Christian syndrome. In *Handbuch der Histochemie V/2 Lipide*, p. 50. G. Fischer Verlag, St ttgart, 1964
20. ÅSTRÖM, K. E. MANCALL, E. L. and RICHARDSON E. P Progressive multifocal leukoencephalopathy *Brain*, *81* 93, 1958.

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CASE REPORT

Chronic Idiopathic Jaundice (Dubin-Johnson syndrome) in Three Sisters

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Chronic idiopathic jaundice, also known as Dubin-Johnson's (Dubin-Sprinz') syndrome is a congenital form of chronic non-hemolytic intermittent jaundice with elevated conjugated bilirubin in the serum and a brown pigment in the liver cells. It belongs to the same group of diseases as Gilbert disease and Rotor's syndrome The three diseases can be differentiated mainly in the light of differences in the morphologic findings and the distribution between conjugated and non-conjugated bilirubin in the serum (Table 1)

The main characteristic of Dubin-Johnson syndrome is a usually slight hyperbilirubinemia of non-hemolytic type with a favourable prognosis. It is most commonly diagnosed in relatively young patients, but can occur in all age-groups. In connection with intermittent attacks of mild jaundice which often appear to have been triggered off by bodily strain or infections, the patients complain of lassitude and uncharacteristic gastro-intestinal symptoms. Hepatomegaly and tenderness of the hepatic region is often found on palpation.

During the attacks of jaundice a hyperbilirubinemia with a positive direct van den Bergh reaction is found, and the thymol turbidity test and bromosulphthalein test are also usually pathological. The urine is darker than normal. On cholecystography the gall bladder and bile-ducts are usually not visualized, or the concentration of contrast is very low and the bile-ducts very narrow

Chronic idiopathic jaundice has a familial tendency Dubin [7] states that of the 30 cases of the syndrome where relevant data on family history were available 13 patient were aware of jaundice in the family and two of those were siblings Other findings with similar implications have been reported [1 –, 3, 9 11 1 14, 15 17 23, –5]

Beker & Read [9] and Mandema *et al* [15] assert that the mode of inheritance is probably dominant, but the reports so far available are very few All statements must therefore be regarded as very uncertain.

In all the cases published since the original observations made independently by

TABLE 1 *Comparison of main features in Gilbert's disease, chronic idiopathic jaundice and Rotor's syndrome.*

	Gilbert disease	Chronic idiopathic jaundice	Rotor' syndrome
Intermittent jaundice	+	+	+
Non-conjugated bilirubin	Elevated	Elevated	Elevated
Conjugated bilirubin	Normal	Elevated	Elevated
Hemolysis	—	—	—
Bromsulphalein test	Normal	Pathological	Pathological
Thymol turbidity test	Elevated	Elevated or normal	Normal
Colour of the urine	Normal	Dark	Dark or normal
Intracellular pigment in the liver	—	+	—
Hepatomegaly	—	+	—
Cholecystography	Normal visualization	Often no visualization or faint visualization of narrow bile ducts	Normal visualization
Familial incidence	Often	Often	Often

Dubin-Johnson and Nelson-Sprinz in 1954 the hepatic changes reported have been consistent. The liver is described as black, dark brown, greenish brown, or bluish black. The surface is smooth and the structure normal. It has been proposed [4] that the condition be called mauro-hepatic icterus (mauros-black)

Microscopically the lobular structure of liver is well preserved. Only in an occasional case was cirrhosis found [4] and this appears to have been coincidental The characteristic histopathologic feature have been collections of pigment granules of various sizes in the parenchymal cells of the liver to a lesser extent also in the Kupffer cells. The pigment has a tendency to be concentrated mostly to the central parts of the lobules. Otherwise the liver parenchyma presents a perfectly normal appearance in the great majority of cases. Slight changes such as fatty metamorphosis of various degrees have been reported in a few cases. These should apparently be interpreted as incidental findings without any definite relation to the pigmentation. According to Wolf [5], the amount of pigment in the same patient varies greatly Dubin [7] on the other hand, declares that "the amount of pigment in the liver does not fluctuate in any given case. Apparently the liver becomes saturated with the pigment which it cannot excrete and retains it even during remissions when jaundice disappears"

Case Reports

In the following an account is given of three sisters, all suffering from intermittent jaundice. Two of them have histopathologic liver changes of the type found in Dubin Johnson syndrome

The parents are first cousins. One paternal and one maternal aunt are reported to suffer from cholelithiasis.

Case 1

Girl born in 1947 Uncomplicated pregnancy and delivery Weight at birth 3500 g Early development normal. Healthy up to 1958, when she was examined in a paediatric hospital on account of headaches. No jaundice was observed on this occasion. Physical examination in 1959 showed slight jaundice



Fig. 1. Case 1. Liver. Low power. Note the concentration of the pigment to the centrolobular areas. Htx. eos. approx. × 80.

TABLE ... *Laboratory data in our three patients.*

	Case 1	Case 2	Case 3
Total serum bilirubin* mg%, highest recorded level	3.2	2.8	3.2
Total serum bilirubin (1964)	1.8	0.32	1.9
Direct reacting serum bilirubin	83 %	0 %	90 %
Transaminase units			
GOT	20	26	14
GPT	27	15	41
Aldolase units	11	6	5
Transferase (OCT) (microg N/ml in 24 hrs.)	0	12	16
Alkaline phosphatase (Buch & Buch units)	3	10	16
Thymol (units)	0	1	2
Takata test	Normal	Normal	Normal
Bromsulphtalein excretion test	Not done	Normal	Not done
Galactose loading test	Normal	Normal	Normal
Hemoglobin g%	12.8	13.7	12.3
Erythrocytes mill./mm³	4.3	4.9	3.9
Reticulocytes %	0.4–1.6	1.0	0.8
Mean erythrocyte diameter μ	7.2	7.3	7.2
Osmotic resistance	Normal	Normal	Normal
Total serum protein g%	7.3	7.3	7.6
Serum electrophoresis	Normal	Normal	Normal
Sedimentation rate	MSR 8 mm/h	SR 3 mm/h	MSR 14 mm/h
Coombs' test	Neg.	Not done	Not done

* Bilirubin was determined according to Jendrassik's method. From 1964, however, the modification of this method by Nosslin (18) and Michaelsson (16) was used.

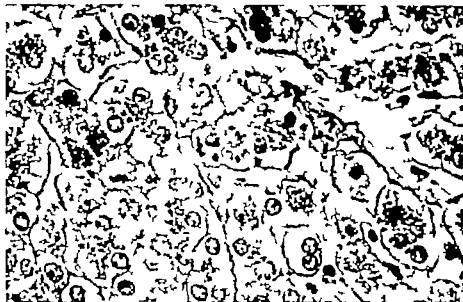


Fig. 2. Case 1. Liver. High power view of hepatic pigment. Htx. eos. approx. × 510.

and the girl was readmitted. She had had mild uncharacteristic gastrointestinal symptoms some days before admission. The serum bilirubin was 2.6 mg/100 ml. No abnormalities were found on palpation of the abdomen. On the suspicion of hepatitis she was sent to the Hospital for Epidemic Diseases. The clinical diagnosis was epidemic hepatitis (juvenile intermittent jaundice?). She has subsequently suffered from repeated irregular attacks of jaundice of varying duration and frequency. The attacks have often been preceded by fatigue which has continued during the attacks in combination with malaise, dyspeptic symptoms, diarrhea, increasingly severe headache and reddish brown urine. At times there has been diffuse abdominal pain mainly on the right side of the abdomen. The symptoms have sometimes been so marked that she has had to stay in bed for some days. The jaundice has varied in intensity, the maximum estimation of total serum bilirubin being 3.2 mg/100 ml. No signs of increased hemolysis and "liver function tests" have proved consistently normal (Table 2). Several attempts at cholecystography by oral administration of contrast were made in 1961 but no visualization of the gall bladder was obtained. On intravenous cholegraphy a rather faint visualization of the gall bladder was obtained after 2 hours, but no definite contrast could be seen in the bile ducts. The patient was referred to the Surgical Clinic of Karolinska Sjukhuset for further examination. An attempt at transhepatic cholangiography was unsuccessful and a laparotomy was performed. The liver was of normal size but very dark. Cholangiography by means of a catheter in the gall bladder disclosed narrow deep bile ducts and the contrast medium passed on to the duodenum. A biopsy was performed (see below).

Case 2

Girl born in 1949. Pregnancy and delivery uncomplicated. Weight at birth 3800 g. Healthy apart from enuresis, for which she was hospitalized in 1955. In 1958 a short attack of abdominal pain and a temperature of 38 C. Otherwise no gastrointestinal complaints and no attacks of jaundice. In 1962 a more thorough examination was carried out after a Dubin-Johnson syndrome had been

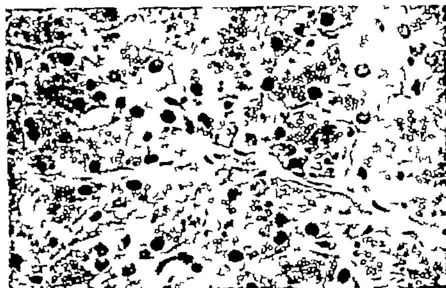


Fig. 3. Case 2. Liver Moderate amount of pigment in liver cells. Htx. eos. approx. 510.

diagnosed in the elder sister Total serum bilirubin was 1.8 mg/100 ml, but four days later it was normal. On physical examination no abnormalities were observed Cholecystography by oral administration of contrast showed normal visualization of a normal gall-bladder Liver function tests were normal (Table 2) A percutaneous liver biopsy was performed (see below).

Case 3

Girl born in 1954. Pregnancy and delivery uncomplicated. Weight at birth 3700 g Healthy up to the age of four when mild jaundice was observed at medical examination undertaken because the eldest sister had been admitted to the Hospital for Epidemic Diseases. She was also admitted t

TABLE 3 *Histochemical reactions of the pigment in the liver*

Histochemical method	Case 1	Case 2	Case 3
Natural colour of pigment	Brown-yellow	Brown yellow	Brown-yellow
Fluorescence	Brown-yellow	Not identified	Brown-yellow
Melanin-stain according to Masson	+	+	+
Lipofuscin-stain acc. to Schmorl	+	( )	+
Periodic Acid Schiff (Hotchkiss)		−	
Trichrome Periodic Acid Schiff (Hotchkiss)	−	−	−
Performic Acid-Schiff method (Pearse)		−	−
Sudan Black B stain (paraffin-embedded material)	−		
Sudan Black B stain (frozen section)		−	−
Scharlach Rot (frozen section)		−	−
Tbb (Ziehl-Neelsen)	−	−	−
"Long" Ziehl-Neelsen method	−	−	
Bile pigment stain acc. to Gmelin	−	−	−
Pigment stain acc. to Glenner-Lillie	−	−	−
Iron pigment stain (ferrocyankalium)	−	−	−

that hospital about a week later than the eldest sister on the suspicion of hepatitis. Bilirubin on admission 2.0 mg/100 ml and ten days later 0 4 mg/100 ml. Li func-tion tests" normal. No signs of increased hemolysis. The clinical diagnosis was epi demi hepat tis (Icterus juvenilis intermit tens?) In the sequel the girl has had mild attacks of jaundice with lassitude and dark urine. In connection with these ttacks some malaise but no real abdominal pain. Control tests were performed in 1962 t which serum bilirubin was 1 4–3.2 mg/100 ml Cholecystography by the oral administra tion of contrast gave a rather thin contrast but otherwise normal findings. A percu taneous li er biopsy was performed (see below)

Histopathologic examination of liver specimens

In cases 1 and 3 the pieces of liver tissue presented a characteristic appearance ven to macroscopic examination. Their colour was very dark. In case 2 the liver tissue had the normal pale brown colour

Microscopically all three cases displayed a normal lobular structure. No cirrhosis or fibrosis. A brown pigment was found in the arenchymal li er cells, abundantly in case 1 and in moderate amounts in case 3. Also in case 2 there were some similar pigment granules, but only in ery small amounts. In this case, too, the pigment showed the same histochemical reactions as in the other two girls. The pigment was clearly concentrated to the central parts of the lobules. The liver cells were otherwise normal. No fatty va cuolization worth mentioning was observed and no inflammatory infiltration. No necroses (Fig 1 to 3).

Histochemical analysis

An attempt was made to characterize the pigment through some simple histochemical reactions. The pigment had f the most part the same characteristics in all three cases (Table 3).

Discussion

In the present sibship there are two certain cases of Dubin-Johnson's syn drome How the condition of the third sister (case 2) should be judged is more debatable She has had one attack of jaundice but otherwise has no clinical symptoms. It might be maintained that she suffers from a very mild form of chronic familial jaundice. The slight pigmentation of the liver cells makes it difficult to decide whether this should be classified as a Dubin-Johnson's syndrome a Rotor's syndrome or an intermediate form

The Dubin-Johnson and Rotor syndromes are characterized by jaundice with an elevated direct serum bilirubin, but there is no pigment in the liver cells in Rotor's syndrome. The familial appearance of the Rotor syndrome was pointed out in the original communication by Rotor *et al* [20] Arias [1] describes three cases in the same family (two brothers and a paternal aunt) all of whom presented the same clinical picture a chronic non hemolytic jaundice with conjugated bili rubin in the serum. One brother and the paternal aunt had pigment in the liver cells and were classified as cases of Dubin-Johnson's syndrome. The other brother had no pathological hepatic pigment and was regarded as a case of Rotor's syn-drome Arias discusses the possibility that both syndromes represent varied ex pressivity of a common functional defect in the poorly understood mechanisms by which bilirubin and other metabolites are transported from the liver cells into the bile"

Possibly our case 2 represents an inter mediate form between these two syndro-

mes. Such an intermediate form might be expected to exist if the theory advanced by Arias is accepted. Dubin [8] on the other hand, is very reluctant to accept intermediat forms between the two syndromes and stresses the importance of ri gidly observing the original criteria of the two diseases.

Concerning the nature of the pigment there are different opinions. The majority regard it as a lipofuscin, though other views have also been recorded. Thus Bynum [4] claims that the pigment is of the melanin type. Wegmann *et al.* [4] claim *to have proved that the pigment* granules are melanin, of the type "adenochrome Sallet *et al.* [1] believe that *the finding* of melanuria in their patient supports this theory Melanuria appears to have been reported only in three cases to date [4, 5, 1] Hamperl [10], who refers the pigment to the group of lipofuscin, is of the opinion that this group of pigments cannot be defined distinctly He claims that the varying results of the histochemical analysis of the pigment are due to different degrees of maturity" of the pigment. Pearse [10] gives a diagram of the histochemical char teristics of ceroid, lipofuscin and its pr ursors According to this, the pigment in the present cases seems compatible with lipofuscin. Histochemical analysis by different workers has thus given very confusing results. The nature of the pigment still seems to be very uncertain.

Summary

Three sisters, aged 18, 16 and 11 years, were suffering from familial jaundice with elevated direct bilirubin. Two of them had a *brown pigmentation of the live cells* and had slight, diffuse gastrointestinal symptoms. They meet the criteria for Dubin-Johnson's syndrome The third suffering from a very mild form of fa milial j undice, had very little pigment in the liver cells. This case is difficult to classify being either a Dubin-Johnson's syndrome a Rotor's syndrome or a condition intermediate between these two Attempts to characterize the hepatic pigment with histochemical methods were made and seem to indicate that the pig ment belongs to the lipofuscins.

Ref rences

1 ARIAS, I. M.: Studies of chronic familial non hemolytic jaundice with conjugated bilirubin in the serum with and without an unidentified pigment in the liver cells. Am J Med, 31 510, 1961.
2. BUTLER, S. and READ A. E. Familial Dubin Johnson syndrome *Gastroenterology* 3 247 1964.
3. BERGQVIST, N Familjar kronisk idiopatisk ikterus. Nord Med, 69 343, 1963.
4. BYNUM, W T Melano-hepatic icterus (black liver jaundice). *Gastroenterology,* 33 97 1957
5. CATTAN R., ACAR, J RABOTEAU, M., JOURNAND, A. and HABIB, R.: Maladie de Dubin t Johnson vec mélanurie *Sem H p Paris,* 36 3019, 1960.
6. DUBIN, I. N and JOHNSON F B.: Chronic idiopathic jaundice with unidentified pig ment in the liver cells. *Medicine* 33 155, 1954.
DUBIN I. N Chronic idiopathic jaundice *Am J Med,* 24 268, 1958.
8. — Rotor's syndrome and chronic idiopathic jaundice *Arch Intern Med,* 110 823, 1962.
9 EHRLICH, J C., NOVIKOFF A. B., PLATT R. and ESSNER, E. *Hepatocellular lipofus*cin and the pigment of chronic idiopathic jaundice. *Bull N Y Acad Med* 36 488, 1960.
10 HAMPERL, H.: Chronischer nichthemolytischer Ikterus mit Ablagerung eines eigentumlichen Pigmentes in der Leber *Klin Wschr* 35 177 1957

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Possibly our case represents an intermediate form between these two syndro-

Acta Pædiatrica Scandinavica 55: 337–340, May [illegible]

CASE REPORT

Achromobacter Septicemia in a Premature

Clinical and Bacteriological Aspects

by LARS Å. HANSON, JAN KJELLANDER and AGNETA LIND JÖ

From the Department of Pediatrics, Kronprinsessan Lovisas Barnsjukhus, the Department of Clinical Bacteriology, Karolinska Institutet and the Central Bacteriological laboratory of Stockholm City, Stockholm, Sweden

The Gram negative bacteria of the Achromobacter group are usually considered as non pathogenic. Only rare cases have been reported where these and closely related bacteria have caused infection, mostly severe fatal infections in new borns [1, 2, 6].

We have observed a case of Achromobacter septicemia in a premature of 1650 g who survived. This case illustrates the potential pathogenicity of these common bacteria and stresses the risk of aerosol infection in the incubator.

Case Report

The patient was a boy born after 30–31 weeks of pregnancy. The mother had proteinuria shortly before delivery and was treated for a urinary tract infection afterwards (infecting organism unknown). The mother was I-gravida and was not immunized.

Birth weight was 1650 g. The boy had weak cry and cyanosis after delivery and was brought to a local hospital. There were atelectatic changes on the lungs. During the first three days of life he was treated with ampicillin. Because of increasing hyperbilirubinemia he was admitted to the Pediatric Clinic on the third day of life. Blood group O Rh (–), direct Coombs test neg, serum bilirubin 23 mg/100 ml. Exchange transfusion was performed. This had to be repeated twice on the next day due to a rise of serum bilirubin to 34 and 28 mg/100 ml respectively. On the fifth day of life bilirubin reached 23 mg/100 ml—a fourth exchange transfusion was performed. After this exchange transfusion the child was in a poor condition with ileus, bronchopneumonia-like changes on X-ray and infections in two of the cut down sites. Treatment with penicillin and streptomycin for eight days was followed by obvious improvement. Blood culture before treatment was negative.

At the age of 2½ weeks, 6 days after treatment was discontinued, attacks of cyanosis recurred. X-ray of lungs was normal. In two blood cultures (three days apart) and in one culture from cerebrospinal fluid (CSF) growth of *Achromobacter* was recorded (see below). Poor vitality, increasing edema in legs and face, finally signs of pulmonary edema were noted. Serum protein 3.7 g/100 ml. Hb 8 g/100 ml. In CSF 208 mg/100 ml of protein and 9 leucocytes/mm. Treatment with oxytetracyclin for 17 days (15 mg/kg and day i.m. for one week, then perorally thereafter sulfa (0.1 g/kg and day). Two blood cultures during therapy were neg. One blood transfusion was given and for one

week daily i.v. injections of 1 g of albumin. Serum protein level stayed around 4.1–4.4 g/100 ml. Injection of albumin –I[131] showed a normal elimination curve for blood and urine indicating that the injected protein was lost extra-vascularly.

Thereafter the boy improved well with normal weight increase and normal development except for a consistently negative auropalpebral reflex bilaterally and poor response on wakening considered due to a neurogenic auditory damage.

Bacteriological and epidemiological investigations

The bacterium isolated from the blood and CSF was a non motile Gram negative rod, which did not ferment lactose, saccharose, mannitol, dextrose, maltose, arabinose, dulcitol, xylose or sorbitol. It did not produce indol or H_2S and was citrate, urease and nitrate negative. Growth without pigmentation was obtained on plain agar at 22°C and 37°C. On the basis of these results it was classified as belonging to the *Achromobacter* group (or *Acinetobacter* according to Brisou & Prevot), a group where the nomenclature is much confused. [5] In vitro the strain was sensitive to tetracycline, oxytetracycline, chlortetracycline, chloramphenicole and kanamycin, but resistant to sulpha, penicillin, ...thromycin and streptomycin.

In order to clarify the route of infection a number of environmental investigations were performed. No growth of *Achromobacter* was obtained from the humidifying water of the incubator where the baby was kept, nor from any other of the incubators in the ward. *Achromobacter* with the same biochemical characteristics as the strain from the blood and CSF cultures was, however, found in a humidifier attached to an oxygen tank which was periodically applied to the incubator.

Eng. Plantm. Gustaf V Forskningsinstitut, Stockholm, is acknowledged for his kind help with these determinations.

Some of the cases of *Achromobacter* infections have been described in the literature as probably being caused by infected aerosols [1, 2]. In order to further investigate this possibility bacteriological investigations were performed using a slit sampler (Casella Ltd.) to study the degree of contamination in an incubator fitted with a humidifier producing an aerosol. One end of a wide rubber tube was attached to the air inlet of the sampler. The other end was attached to the inside of the incubator in one series of experiments and to the air outlet of the incubator in another series.

When the first sampling was performed the water in the humidifier had not been changed for 24 hours. An air count of approximately 200 bacteria per cubic foot was obtained both from the inside and the air outlet of the incubator. The organisms belonged to the *Hafnia* group of bacteria. There was also an abundant growth of the same organisms in the humidifying water.

By the time of the next sampling the water had been changed 3–4 hours earlier. This time 38 organisms per cubic foot were obtained from the incubator and 40 from its air outlet. The same organisms were cultured from the humidifying water although less abundant than in the previous sample.

In a third series the humidifying tank was emptied, soaked in a 2 % chloramine solution and rinsed thoroughly before being filled again with water and used. Three to four hours after this procedure an air sample showed growth of less than one organism (*Staph. albus*) per cubic foot both from the incubator and its outlet. No bacteria of the previously described

type was demonstrable in the air or in the humidifying water

Discussion

Septicemia and/or meningitis in the neonatal period is often caused by Gram negative bacteria, especially coliforms. It has been found, however that in this age group infections can also be caused by bacteria usually presumed to be nonpathogenic. The etiology of these infections can easily be overlooked since the organisms cultured are often considered as contaminants. Cases reported in the literature [1 2, 6, 7] mostly had a fatal outcome. Probably due to the effectiveness of the oxytetracyclin therapy against the actual organism our case survived in spite of a high degree of prematurity and an alarming clinical picture.

Of possible routes of infection the aerogenic is the most probable in this case as well as in some earlier reported [1 –, 7]. The same organism was cultured from the humidifying water for the oxygen apparatus as from the patient's blood and CSF The experiments using the slit sampler well demonstrates the high bacterial content of the air in an incubator fitted with a similar humidifying apparatus. If the water used is contaminated the patient is continuously exposed to the large dose of bacteria necessary to induce an infection with these bacteria of low virulence [4].

The only way of av iding such incidents is to prevent contamination of the water used for humidification The effectiveness of such a measure was illustrated by the experiment in which the air contamination was reduced to practically nil by disinfecting the water container with chloramine. It is our experience however that the humidifiers of most types of incubators are so poorly constructed that it is quite laborious or even impossible to clean them effectively

Other possible routes of infection should also be considered. For instance unboiled breast milk was shown by Klinge [3] to contain faecal or pathogenic bacteria in 84%. Among these organisms the most commonly found was *Achromobacter* which was present in 01 of the milk samples. We have not however been able to isolate *Achromobacter* from samples of unboiled breast milk.

Summary

A case of *Achromobacter* septicemia in a premature is described. This normally non pathogenic organism probably infected the infant through an aerosol from an infected humidifier Bacteriological sampling shows that such an aerosol can be heavily contaminated. Disinfection f the humidifier is shown to be an effective preventive measure However the water containers of most incubators are quite difficult to disinfect effectively

References

1. Editorial: Water bugs in the bassinet. *Am J Dis Child, 101* 73, 1961
2. Foley J F Gravelle, C R. and Englehard W E: Achromobacter septicemia fatalities in prematures. I: Clinical and epidemiological study *Am J Dis Child, 101* 279 1961
3. Klinge, K.: Die Bakterienflora ungekochter

Frauenmilch. *Münch Med Wschr 104* 1764, 1962.
4. PAINE, T Illness in man following inhalation of *Serratia marcescens*. *J Inf Dis, 79* 226, 1946.
5. PICKETT M. J and MANCLARK, C. R Tribe Mimeae. An illegitimate epithet. *Am J Clin Path, 43* 161 1965.
6. PONGLIONE, R. and JANNUZZI, C.: Endocardite letale da *Achromobacter* in bambino affetto da stenosi valvolare aortica congenita. *Minerva Ped, 16* 25, 1964
7 UMMERY A. M. C. and FRANKLIN A. W Neonatal death from pigmented coliform infection. *Lancet, I* 313, 1961.

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CASE REPORT

Congenital Alkalosis with Diarrhea

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Congenital alkalosis with diarrhea is a rare disorder of which in all ten cases have been described in the literature by Gamble *et al.* [4] Darrow [1], Kelsey [5], Duyck [2], Perheentupa *et al* [8], Tucker *et al* [9], and Owen [7] The disease is characterized by watery diarrhea with high chloride concentration, persistent metabolic alkalosis, and a severe disturbance of the electrolyte metabolism. The alkalosis and diarrhea persist, even when the serum electrolytes have been restored to normal by adequate therapy We have had the opportunity to study a newborn male infant with this rare disorder

Case History

Our patient was the second child of healthy parents; two siblings were healthy There was no family history of similar symptoms or other metabolic disorders. The pregnancy was complicated by hydramnios, and the child was born six weeks before term. The birth weight was 2450 g. It was immediately noticed that the abdomen was distended, and because the child passed watery stools during the first few hours of life, he was referred t this clinic with a tentative diagnosis of congenital hydronephrosis with ectopic ureters. H continued to excrete a watery fluid containing particles of yellow feces rectally but typical meconium was not observed. After three days of observation an exploratory laparotomy was performed. No gross malformations were found, the kidneys and urinary tract were normal, but the intestines and colon were dilated, containing air and watery fluid. Roentgen examination f the colon was carried out later and showed nothing abnormal. As low serum concentrations of sodium (117 mEq/l) and chloride (65 mEq/l) were found, the child was put on human milk, and 2 g f sodium chloride and extra glucose-water were given daily Later potassium chloride was added. The subsequent values f his serum electrolytes are shown in Fig. 1 and 2.

The course of this patient was typical of this disorder presenting the paradoxical combination f persisting watery diarrhea and alkalosis. The serum pH remained elevated between 7 40–7.55, even when the serum electrolytes had been corrected during treatment. Serum sodium was maintained within normal limits for long periods, whereas serum potassium tended t decrease below normal values, and serum chloride remained at subnormal levels between 90–100 mEq/l, except for short periods. Standard bicarbonat and pCO fluctuated around normal values.

Different treatments were tried as discussed below During the first months the child had signs of cerebral irritation with rigidity hyperactive reflexes and lonus of the feet. Chvostek sign was negative

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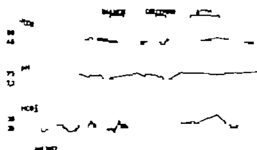


Fig. 1. Serum pCO_2, pH and bicarbonate values during the period January to October 1962.

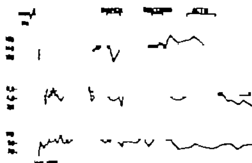


Fig. 2. Serum sodium, potassium and chloride values during the period January to October 1962.

and no attacks of tetany were observed. The symptoms disappeared gradually but his motor and mental development became retarded. At age ten months he was acutely ill with measles complicated by otitis, and at twelve months his condition continued to be unsatisfactory. His diarrhea exacerbated, and he was readmitted with dehydration and severe shock, from which he succumbed after a few days in spite of intensive treatment.

Observation

Different treatments were tried. First acetazolamide (Diamox) was given for two weeks without any change in the serum electrolytes or acid-base balance of the blood, except for a temporary fall in sodium and potassium. Next the child was put on prednisone for nine days, during which period the serum pH showed a tendency to decrease while the serum chloride increased slightly but similar values had previously been observed without steroid treatment. Serum sodium and potassium remained unchanged. Later on ACTH was given for one month (Acton prolongatum 20 I.U. daily) without any effect on the serum electrolytes.

Serum magnesium was normal at 1.8–2.4 mEq/l. The sodium and potassium concentration in the erythrocytes were normal, and a glucose tolerance test was likewise found to be normal.

The daily stool volume varied between 100–200 ml with a pH of 5.2 to 6.5 and a chloride concentration between 121–171 mEq/l. This concentration is exceedingly high as compared to that found in ordinary diarrhea, as is shown in Fig. 3. Furthermore the chloride concentration was found to be constantly higher than the sum of the sodium and potassium ions, which again is in contrast to the findings in simple diarrhea, where the concentration of chloride usually is lower than this sum. Great variations were found from day to day in the cation excretion so that either sodium or potassium was found to be the predominant ion. No change in the composition of the stools was observed during steroid treatment.

The pH of the urine varied between 5.7 and 7.3 and usually no chloride could be detected in the urine. On a few occasions when the serum chloride was within normal range, a chloride concentration of 2–7 mEq/l was found in the urine. Treatment with either prednisone or ACTH did not produce any excretion of chloride in the urine, but

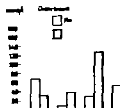


Fig. 3. The concentration of sodium and chloride in fecal water (*F*) and urine (*U*) in a patient with ordinary diarrhea and our patient (H. N.).

after withdrawal of steroid treatment a trace of chloride appeared in the urine, almost as a rebound effect. Sodium and potassium in the urine were found to vary from 10 to 200 mEq/l.

His gastric secretion was examined after injection of betazole chloride (Histalog®) whereby a normal secretion of gastric juice was found. The pH changed from 7.0 (fasting) to 2.0, and the chloride concentration increased from 82 to 102 mEq/l. 10 ml of gastric juice were collected during a 15-minute period, which was considered to be within normal limits.

When Secretin was given (7 I.U.) i.v., a duodenal sample was obtained with a high pH (8.3), but the composition of the pancreatic secretion showed a low concentration of bicarbonate (?6 mEq/l).

In order to rule out any possible abnormality of his intestinal function a tube was passed, and by roentgen examination was estimated to be in the lower end of the ileum. Samples of intestinal fluid were collected. The chemical composition of these samples was found t be similar to that of the stools with a rather high chloride concentration from 134–142 mEq/l and a pH between 6.4–7.3.

Discussion

Several theories have been put forward to explain this rare metabolic disorder Darrow [1] postulated that a specific defect of chloride absorption from the gut might explain the condition of his patient Gamble [4] on the other hand believed that an active secretion of chloride into the gut lumen was a more probable explanation. Such an excretion could be caused by ectopic gastri mucosa, but the autopsy of his patient failed to show any gross or microscopic abnormality of the intestinal tract As a third possibility Kelsey [5] proposed that the condition was caused by a chronic potassium deficiency with a hypokalemic alkalosis. None of these theories has been able to explain the elevated serum pH or the lack of chloride in the urine even during the periods with normal serum electrolytes.

A very extensive investigation of this condition has been carried out by Duyck [–]. He showed that chloride can be absorbed from the gastro-intestinal tract because the decreased serum chloride in his patient was restored to normal by oral administration of sodium chloride Furthermore he showed that an increase of the concentration of the serum chloride was followed by an increased output of chloride in the stools, regardless of whether the serum chloride had been corrected by giving chloride by enteral or parenteral route. This supports the excretion theory of Gamble but could also be explained by an increased exeretion with a faulty absorption of chloride In both cases a congenital error of metabolism at the cellular level must undoubtedly exist as already pointed out by Gamble and Duyck.

This theory of a specific metabolic defect of chloride metabolism is further supported by recent studies of Kinney & Code [6] who found that the absorption of chloride from the ileum in dogs seems to be accomplished partly by an active transport Thus, a defect of this transport mechanism is possible. It is still not clear however in which way the lack of chloride in the urine is related to the elevated chloride concentration in the stools.

The disease may exist in varying degrees, as indicated by the observation of Owen [7] of a boy who apparently recovered by ½ years of age.

Summary

A case of congenital alkalosis with diarrhea has been described in a newborn male infant. The characteristic finding in the disease is the combination of persistent metabolic alkalosis and watery stools with a high concentration of chloride. The disorder was presumably present already during fetal life and manifested itself from the neonatal period. In spite of treatment the patient died when twelve months old.

References

1 DARROW D C.: Congenital alkalosis with diarrhea. *J Pediat*, *26* 519 1945.

2. DUYCK, E. M.: *L'alcalose congénitale avec diarrhée.* Thesis. Stenfert Kroese, Leiden, 1955.

3. — *Die kongenitale Alkalose mit Diarrhoe Kongenitale Störungen des Wasser und Elektrolythaushalt* Springer Berlin–Gottingen–Heidelberg, pp. 129–140 1962.

4. GAMBLE, J L., FAHEY K. R., APPLETON J and MACLACHLAN E.: Congenital alkalosis with diarrhea. *J Pediat*, *26* 509 1945.

5 KELSEY W M. Congenital alkalosis with diarrhea. *Am J Dis Child*, *88* 344, 1954.

6. KNICKY V R. and CODE, C. F Canine ileal chloride absorption: Effect of carbonic anhydrase inhibitor on transport. *Am J Physiol* *207* 998, 1964

7 OWEN G M.: Metabolic alkalosis with diarrhea and chloride-free urine. *J Pediat*, *65*, 849 1964.

8. PERHEENTUPA, J., EKLUND J and KOJO, N Familial chloride diarrhea (congenital alkalosis with diarrhea), Proceedings of the Fourteenth Northern Pediatric Congress. *Acta Paediat Scand*, *Suppl 159* 1965.

9 TUCKER, V L., WILMORE, D KAISER, C. J and LAUER, R. M.: Chronic diarrhea and alkalosis. *Pediatrics*, *34* 601 1964

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CASE REPORT

Neonatal Ascites and Urinary Tract Obstruction

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Neonatal ascites associated with obstruction of the lower urinary tract was first described by Hicks in 1864 and by Fordyce [3] in 1894. Since then, reports have been published on about 30 cases, most of which terminated fatally. As the disease is thus fairly rare and may give rise to diagnostic difficulties, an additional case is reported below. Once the diagnosis has been made, the treatment usually does not present any great problems, but it is certainly of vital importance to the patient.

Case Report

The patient was a newborn girl, the younger of two children. The mother aged 26, was in good health during the pregnancy and delivery occurred at term without any complications. At birth, the infant was crying normally and there were no signs of congenital disease. Birth weight, 2900 g; length, 50 cm. The infant was apparently in good health until the 12th day of life when she became restless, irritable and began to vomit. The vomitus did not contain bile or blood.

During the next 24 hours, no urine was voided, and the mother noticed that the abdomen of the baby became distended. Two days later the baby was admitted to a local hospital. Considerable oedema of the abdominal wall and signs of ascites were now present. Radiography of the colon with barium enema was suggestive of the presence of ascites, but did not reveal any other abnormalities. On catheterisation, the bladder was found to be empty. As anuria persisted, the patient was transferred to the Department of Paediatrics, Odense County and City Hospital, three days later.

On admission, physical examination revealed an acutely ill, greyish pale, weakly crying female baby. There was no dyspnoea, cyanosis or jaundice. The baby was emaciated and moderately dehydrated. The abdomen showed considerable distension with a prominent venous pattern, slight periumbilical meteorism and massive dullness in the dependent parts of both flanks, lumbar regions and the anterior abdominal wall.

There was no oedema around the eyes or on the extremities. Signs of cardiac disease were not observed. The size of the liver could not be assessed because of the ascites. On admission, the child refused to take its feedings. Complete anuria was present.

Laboratory investigations showed haemoconcentration with a haemoglobin level of 150%, blood urea 216–356 mg/100 ml. Moderate hyponatriaemia, hypochloraemia and slight metabolic acidosis were present. The amounts of serum proteins were normal, and electrophoretic analysis showed a normal distribution.

Paracentesis was performed, with aspira-

TABLE 1 *Twelve cases of neonatal ascites and urinary tract obstruction.*

Sex	Ref.	Age on admission	Symptoms and signs	Lesion	Surgical treatment	Survival
M	[2]	2 weeks	Anorexia, drowsiness, abdominal swelling, normal voiding	Urethral obstruction	Obstruction removed	Well-being at 8 months
M	[5]	5 weeks	Vomiting, loss of weight, ascites, oedema, normal voiding	Urethra not examined	None	Died at 6 eeks
M	[5]	At birth	Ascites, abdominal swelling	Urethral valve	None	Died t 1½ hours
M	[4]	6½ weeks	Vomiting, diarrhoea, abdominal swelling, ascites, normal voiding	Urethral valve	None	Died at 7 weeks
M	[4]	1 day	Abdominal swelling, ascites, anuria	Urethral alve, bladder perforation	Cystostomia, repair of the bladder	Died t 7 weeks
M	[6]	4 weeks	Vomiting, ascites, abdominal swelling normal voiding	Urethral valv	Resection of the valve	Well-being at 4 years
M	[7]	16 days	Abdominal swelling, ascites, oedema, oliguria, anuria	Bladder perforation, no urethral valve	Repair of the bladder	Died at 19 days
F	[1]	At birth	Abdominal swelling, anuria	Vesical neck valve, bladder perforation	Resection of the valve	Well being at 8 months
M	[8]	Stillborn	Ascites, oedema	Urethral val e	—	—
M	[8]	At birth	Ascites, oedema	Urethral valve	Cystostomia	Died t 21 days
M	[8]	3 weeks	Vomiting, abdominal swelling, oliguria	Urethral valve	Cauterisation	Well-being at 2 years
M	[8]	4 weeks	Gastroenteritis, abdominal swelling	Urethral valve	Cauterisation	Well-being after operation

tion of 150 ml of a straw-coloured fluid, which in colour and composition corresponded to ascitic fluid. Ascites re-accumulated rapidly; repeat paracentesis the next day yielded 100 ml of typical ascitic fluid.

The various laboratory findings were not suggestive of hepatic disease. There were no signs of peritonitis or other inflammatory conditions. The body temperature was normal. The condition was interpreted as being due to obstruction of the lower urinary tract. As catheterisation had revealed an empty bladder the anuria could not be due to a urethral valve; the obstructive lesion was to be found somewhere in the ureters.

On the second day in hospital, cystoscopy was performed. The vesical mucosa was found to be normal. It was not possible to find the right ureteral orifice whereas the left orifice was normal. A catheter was inserted into the left ureter which resulted in immediate passage of urine. During the next few days, the urinary output was about 20 ml per hour.

Fluid was infused through a cranial ein. Within two days the fluid balance was restored and, at the same time, the ascites and oedema disappeared completely. As assessed by the blood-urea level, the renal function was normal.

Direct pyelography was performed through the ureteral catheter. This revealed considerable hydronephrosis and hydroureter. The ureter seemed to be stenosed at a level of about 3 cm above the orifice. Intravenous pyelography performed the next day showed satisfactory excretion on the left side, but the renal pelvis and ureter were dilated. The passage through the ureteral catheter was fairly good. There was no excretion on the right side.

After the lapse of 6 days the ureteral

catheter slipped out but urinary output remained unchanged.

Gradually the condition of the baby became fully satisfactory. She took her feedings regularly and did not vomit. The electrolyte balance, renal function and urine analyses showed normal conditions.

Ten days after admission cystoscopy was repeated. This time a normal ureteral orifice was found on the right side but a catheter could be inserted for only about 2 cm. Accordingly bilateral ureteral stenosis was suspected. As this would involve the risk of a renewed occlusion of the left ureter, the infant was transferred to the Department of Surgery.

Operation (Dr. C. M. Madsen) revealed a normal vesical mucosa and ureteral orifices of normal position and appearance, but the right ureter only consisted of a stump measuring 2–3 cm. The right kidney was absent. The left ureter was tortuous and dilated, and stenosed in its intramural course. Resection of the distal 3 cm of the left ureter was performed, with reimplantation of the ureteral stump. The postoperative course was uneventful, there were no signs of ascites, and the child was discharged in well-being.

When the girl was 8 months old, pyuria developed, for which reason she was readmitted to hospital at the age of 9 months. Slight fever had been present on two or three occasions, but this had been ascribed to a common cold. Otherwise, no abnormalities had been noticed. The appetite was good, and the patient had gained weight and developed normally. Physical examination revealed a normal infant, who seemed to thrive well. The general condition was good; weight 7.1 kg; length, 66 cm. There was no anaemia, blood urea and serum creatinine showed normal values. Slight bacteriuria and moderate pyuria were present. Culture of urine yielded growth of *Staph. aureus* and *Esch. coli*.

Intravenous pyelography showed normal excretion on the left side and unchanged hydronephrosis and hydroureter. There was no excretion on the right side. Cystography showed reflux for a fairly long distance up the left ureter without any signs of stenosis, and emptying of the bladder was normal.

The urinary tract infection was easily controlled by antibiotics and the patient was discharged in well-being and with sterile urine. When last seen, at the age of 1– months, she was still doing well.

Discussion

Non-chylous foetal and neonatal ascites occurs in association with various diseases, among which syphilitic cirrhosis of the liver was formerly the most well known, but nowadays this disease is scarcely a significant cause of neonatal ascites. The condition may be seen in association with hepatic cirrhosis due to other causes or as an accompaniment to porto-hepatic anomalies. It may be encountered in peritonitis referable to various causes, including foetal meconium peritonitis associated with meconium ileus. Incompensated cardiac disease may also give rise to ascites, which is then usually also accompanied by peripheral oedema. A relative frequent cause is malformation of the abdominal organs, especially anal atresia of a varying degree of development.

Neonatal ascites in association with urinary tract obstruction is a rare condition. However, it was described as early as 1864 by Hicks and in 1894 Fordyce [3] collected 63 cases of foetal and neonatal ascites, some of which had occurred in association with anomalies of the intestinal and urogenital tract. In 1953 Lord [5] reviewed the literature from 1903 to 1952 inclusive and found a total of 29 cases of foetal ascites, including 18 in which urinary tract obstruction was also present.

At the same time she described two cases of her own, in which foetal ascites was associated with hydronephrosis and hydro-ureters. The most important data from twelve cases of neonatal ascites and urethral obstruction published in the literature since 1952 are tabulated in Table 1

The cause-and-effect relationship between obstruction of the urinary tract and the formation of ascitic fluid is obscure. Obstruction cannot be the only factor at work, since bilateral hydronephrosis and megalo-ureters in the newborn, unaccompanied by ascites, have been described in numerous cases. But in several other cases, including the one reported in this paper there was a distinct causal relation, since the ascites disappeared as soon as the obstruction was relieved and urinary outflow restored. As ascites did not develop until on the 12th day of life complete obstruction cannot have been present at birth. It may be assumed that as the urinary output increased, the dilated and tortuous ureter had formed a kink which ultimately resulted in complete obstruction.

Lord [5] postulated that there must be [illegible]-ct communication between the dilated urinary tract and the peritoneal cavity but she did not succeed in demonstrating such a communication. Marx & Dale [6] also failed to disclose a direct communication Neither a dye injected intravenously nor a radiopaque material injected into the urinary tract could be recovered from the ascitic fluid. Later Swain *et al* [8] succeeded, by means of pyelography in demonstrating the passage of contrast medium from the renal pelvis either to the perirenal or the subcapsular space. In one case the dye could be demonstrated in the ascitic fluid 5 minutes after it had been injected into the bladder

The most reasonable explanation seems to be that a transudation of fluid occurs from the distended urinary tract to the peritoneal cavity This fluid possibly has an irritant effect on the peritoneum, resulting in an increased production of ascitic fluid. The exchange between the ascitic fluid and the plasma occurs so rapidly that it cannot be expected to be possible to demonstrate admixture of urine in the ascitic fluid.

According to Marx & Dale [6], up to 1960 only two cases of neonatal ascites associated with urinary tract obstruction in which the patients survived operation were on record. Later Swain *et al.* [8] reported two cases with up to 2 years survival after operation. Baghdassarian *et al.* [1] described a case in which the patient was alive and well at the age of 8 months. Of the 1? cases of neonatal ascites and urinary tract obstruction mentioned in Table 1 11 were boys. Six of them were not subjected to operation and died shortly after birth. The other six were all operated on, and in five a urethral valve was disclosed. These five children all survived the operation, while the sixth child died a few days postoperatively because respiratory arrest occurred. Neither at operation nor at autopsy was it possible to demonstrate obstruction of the dilated lower urinary tract.

Our patient thus represents the sixth case in which postoperative survival was achieved and it is presumably the only case with unilateral ureteral stenosis and renal agenesis on the other side.

Summary

A case is reported in which ascites and anuria due to unilateral ureteral stenosis and renal agenesis on the other side developed in a 14-day-old girl. After relief of the stenosis, the ascites disappeared, and one year after the operation the patient is alive and well.

References

1. BAGHDASSARIAN, O. M., KOEHLER, P. R. and SCHULTZE, G.: Massive neonatal ascites. *Radiology 76* 586, 1961.
2. DAVIS, J. A. and JAMES, U.: Congenital urethral obstruction presenting as ascites in neonatal period. *Proc Roy Soc Med, 45* 401, 1952.
3. FORDYCE, W.: Intra-uterine ascites, its obstetrical significance and pathology. *Teratologia, 1* 61, 1894.
4. FRANCK, N. E. and BACK, E. H.: Neonatal ascites associated with urethral obstruction. *Arch Dis Child 29* 565, 1954.
5. LORD, J. M.: Foetal ascites. *Arch Dis Child 38* 398, 1963.
6. MARX, K. and DALE, W. A.: Neonatal ascites and obstructive uropathy. *Pediatrics 27* 29, 1961.
7. PERROT, R., BARCAT, J., DUBOIS, J. P. and DEPONDT, M.: Un cas d'ascite néonatale par malformation des voies urinaires. *Arch Franc Pediat, 18* 620, 1961.
8. SWAIN, V. A. J., TUCKER, S., STEPHLER, L. and FRANCK, N. E.: Perinatal ascites due to extravasation of urine from ruptured kidneys. *Clin Pediat, 4* 190, 1965.

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CASE REPORT

Primary Cold Injury in the Newborn

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Low environmental temperature is considered the primary etiologic factor of the clinical syndrome of neonatal cold injury; other factors such as prematurity, brain injury, asphyxia, infections, congenital heart disease, malnutrition and other debilitating states definitely contribute to its occurrence [2, 3, 8, 9].

Most cases of cold injury in the newborn have been reported from Great Britain [2–4, 7–9, 10, 12]. The case reported below is to our knowledge the first to be published from Greece, where the syndrome of neonatal hypothermia is probably occasionally encountered, although mild temperatures predominate throughout the year.

Case Report

A male infant weighing 2850 g was born at term, after an uncomplicated pregnancy and a normal spontaneous delivery. Following discharge from the nursery the baby was swaddled and kept in a large room heated by a wood-fire during the day time only. At the age of 13 days he was noted to have very cold skin, was apathetic and refused his feedings. Symptoms persisted and edema of the face, hands and feet developed during the following three days. During this period of time minimal environmental temperatures were 5.2 to 7.7°C with relative humidities ranging from 56 to 81 per cent.

On admission to the hospital at the age of 16 days the patient gave the impression of a "frozen baby". He had no spontaneous activity and no reflexes could be elicited. The face and distal extremities were deep red in color and edematous. Rectal temperature was 32°C. No other abnormal findings were present. All laboratory values were within normal limits except for an elevated blood urea (76 mg/100 ml). Cultures were sterile.

The patient was immediately placed in an Armstrong incubator which was kept at a temperature of 30 to 32°C. Rectal temperature rose to normal levels within 12 hours at which time the patient started having convulsions of the trunk and all extremities. Subdural and lumbar punctures were normal; laboratory values were within normal limits as well, including a blood urea of 36 mg/100 ml, serum calcium of 9.2 mg/100 ml and blood sugar of 97 mg/100 ml, roentgenograms of the head and chest, an electrocardiogram and an electroencephalogram were negative. Convulsive seizures ceased within 48 hours. All symptoms receded slowly; activity returned to normal and an increase in weight was noted following the 6th hospital day. The patient was discharged 23 days later in an apparent excellent condition of health.

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Comment

Increased rate of heat loss and a limited capacity of heat production may lead to inability of the newborn infant to maintain body temperatures at the adult level, whenever ambient temperatures are low [1 5, 6]. This may explain the occurrence of the syndrome of neonatal hypothermia even at moderately low environmental temperatures. Moreover shivering as an effective means of heat production beyond the first two weeks of life [6] can be abolished by swaddling, a common practice in Greece.

Our patient had a series of generalized convulsions during the phase of rewarming. This complication is not a common incident of neonatal hypothermia. It occurred in one patient only out of a series of 70 patients reported by Bower *et al.* [3]. There seems to be a definite correlation between convulsions and rapid rewarming of these infants [8–10]. It is postulated that rapidly rising skin temperatures, a condition commonly occurring during rapid rewarming, may lead to a skin–core temperature gradient (the skin temperature being higher). This can have an adverse effect on the infant, the mechanism of which is not known [11]. Elevated skin temperatures cause peripheral vasodilation through axon reflexes, thereby increasing blood supply to peripheral tissues. It is possible that under increased skin temperatures the oxygen dissociation curve shifts to allow for an increased oxygen consumption by peripheral tissues, at a time when inadequate cardiorespiratory adjustments under the persisting low core temperatures cannot provide enough oxygen to reverse cerebral anoxia. Convulsions may follow increasing anoxia of the brain.

Summary

The well recognized syndrome of primary cold injury or neonatal hypothermia was seen in a full-term healthy newborn infant, apparently not exposed to extremely low levels of temperature and in the absence of any other intrinsic precipitating factors. Rewarming of such infants should be gradual, otherwise convulsions might be induced.

References

1. Adamsons, K. J., Gandy, G. M. and James, L. S. The influence of thermal factors upon oxygen consumption of the newborn infant. *J Pediat, 66* 495, 1965.
2. Arneil, G. C. and Kerr, M. M.: Severe hypothermia in Glasgow infants in winter. *Lancet II* 276, 1963.
3. Bower, B. D., Jones, L. F. and Weeks, M. M. Cold injury in the newborn. A study of 70 cases. *Brit Med J I* 303, 1960.
4. Braithwaite, J. V. Hypothermia in the newborn. *Lancet I* 772, 1958.
5. Brück, K. Temperature regulation in the newborn infant. *Biol Neonat, 3* 63, 1961.
6. — General aspects of temperature regulation of small subjects. In: *The Adaptation of the Newborn Infant to Extra-Uterine Life.* Nutricia Symposium, Leiden, Holland, 1964, pp 229–246.
7. Farquhar, J. W. Neonatal cold injury. *Lancet, I* 371 1951.
8. Mann, T. P. Hypothermia in the newborn. A new syndrome? *Lancet, I* 613, 1955.
9. Mann, T. P. and Elliott, R. I. K.: Neonatal cold injury due to accidental exposure to cold. *Lancet I* 229 1957.
10. Oppé, T. P. and [illegible] K.: Neonatal cold injury. *Lancet I* 327 1957.

11 ... VERMAN, W. Iatrogenic aspects of ther... regulation. In: *Thermoregulation of the ... ly Born*, Supplement to reports of ... conferences on Pediatric Research, ...olumbus, Ohio, 1962, pp. 31

1... SMALLPEICE, V. Hypothermia in the new born. *Lancet*, *I* 679, 1955.

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The Pattern of Blood Lipids Glycerol and Ketone Bodies during the Neonatal Period Infancy and Childhood

by BENGT PERSSON and JOHAN GENTZ

Since protein catabolism can account for only a small fraction of the calorie requirement during the first days of life, the newborn infant is dependent on its stores of glycogen and fat The carbohydrate reserves are consumed rather rapidly and the steady drop in respiratory quotient beginning within some hours of birth indicates an increasing oxidation of fat. Lipolysis of triglycerides stored in adipose tissue produces free fatty acids (FFA) and glycerol, the molar ratio being 3 1 if the hydrolysis is complete. However only part of the FFA are released to the bloodstream bound to albumin, the rest could either be reesterified in the adipose tissue with α glycerophosphate derived from glucose or be oxidized in the adipose tissue.

The released glycerol, on the other hand, is poorly utilized by the adipose tissue due to lack of glycerokinase [37] and therefore the amount of glycerol released could be taken as a measure of the rate of lipolysis.

It has previously been demonstrated that the plasma levels of FFA rise rapidly during the first hours of life [11]. It is also established that the total lipids, cholesterol and phospholipids increase during the first days of life [1–, 4 27] The aim of the present investigation was to study the levels of FFA, glycerol and ketones in full term infants during the period of postnatal adaptation and to compare them to levels in older children. Included in the study has been an estimation of cholesterol, phospholipids and glycerides. The latter lipid fraction was included because of the close metabolic relationship between FFA and glycerides.

Material

A total of 272 enous blood samples was obtained from umbilical cord and from children of varying ages from birth to 8 years. All were analysed for blood glucose and either FFA, glycerol or ketone bodies. In 133 subjects blood glucose, FFA and glycerol were simultaneously determined.

In 61 subjects blood glucose, FFA and the remaining blood lipids (cholesterol phospholipids and glycerides) were analysed simultaneously

The subjects are divided into the following age-groups cord blood, 5–24 hours, 1 2 days, 3–9 days, 1–6 months, 1–8 years, 0– 4 hours, 2–3 days, 4–5 days and 6–7 days. The number of subjects in each age group, and the parameters studied are shown

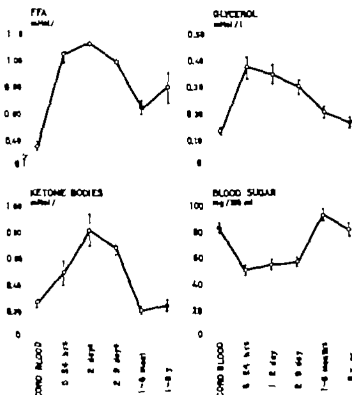


Fig. 1. FFA, glycerol, ketone bodies and glucose in different age-groups (mean values ± error of the mean).

in Tables 1 and 2. The infants, 0–9 days of age were mature and considered normal. They all remained healthy during the neonatal period. After uncomplicated pregnancies they were delivered vaginally in a room temperature of approximately 22–24°C and were placed in beds within 15 minutes after delivery.

Cord blood was obtained in the following manner: Immediately after delivery the cord was clamped by two forceps, placed close to one another and the cord divided between them. From the placental stump of the umbilical cord the blood was allowed to flow spontaneously into a test tube.

Feeding was not yet started in infants of 0–24 hours age group but in the other groups breastmilk had been given in varying amounts. The blood samples were drawn from the internal jugular vein at 3½ hours after the preceding meal. In seven full term infants repeated analyses for blood glucose, FFA and glycerol were done. Blood samples were obtained from cord blood and at one or two times from the internal jugular vein during the first two hours of life (Fig. 3). Noradrenaline (0.2 µg/kg/min. during 15 min.) was infused to one full term infant of 12 hours age. The environmental temperature was kept between 32 and 33°C during the infusion and blood was drawn at intervals for analyses of FFA, glycerol and glucose (Fig. 4).

The older age groups comprise normal children without signs of metabolic disorders. Venous blood was drawn from a cubital vein after an overnight fast. All blood samples were kept on ice until analysis, which was commenced within 4 hours on all samples except cord blood where the interval was up to 12 hours.

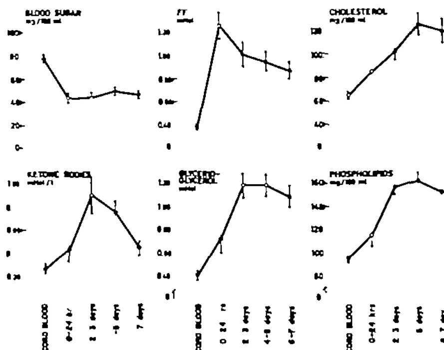


Fig. 2. FFA, glucose, ketone bodies, cholesterol, phospholipids and glycerides in different age-groups (*mean values ± error of the mean*).

Table 1 *Mean blood values ± error of the mean for FFA glycerol, ketone bodies and glucose of full term i fants and children n different age groups*

The figures in brackets refer to the number of subjects in each group. Degree of statistical difference: $p < 0.001$ $p < 0.01$ – $p < 0.1$ –

Age-group	FFA mmol/l	p	Glycerol mmol/l	p	Ketone bodies mmol/l	p	Glucose mg/100 ml	p
Cord blood	0 38 ± 0.016 (41)		0.142 ± 0.006 (24)		0.37 ± 0.04 (22)		63 ± 3 (45)	**
5–24 hours	1 04 ± 0.060 (28)		0.387 ± 0.026 (29)		0.49 ± 0.09 (12)		52 ± 3 (33)	
1 2 days	1 12 ± 0.085 (21)	—	0.350 ± 0.027 (19)	—	0.82 ± 0.13 (17)		33 ± 3 (24)	—
2–9 day	0.99 ± 0.056 (34)	—	0 301 ± 0.019 (28)	—	0.60 ± 0.06 (42)	—	58 ± 2 (47)	—
1–6 months	0 68 ± 0.051 (28)		0.207 ± 0 011 (28)		0.21 ± 0.03 (26)		90 ± 2 (28)	
1 2 years	0.80 ± 0.107 (15)	—	0.179 ± 0 014 (18)	—	0.[illegible]4 ± 0.04 (15)	—	82 ± 4 (18)	

TABLE 2. *Mean blood values ± error of the mean for FFA glycerides phospholipids cholesterol ketone bodies and glucose in full term infants during the first week of life.*

The figures in brackets refer to the number of subjects in each group. Degree of statistical dif ference: $p < 0.001$ – $p < 0.01$ – $p < 0.1$ – Highly significant differences ($p < 0.001$) occur between cord blood and age group 2–3 days for glycerides, phospholipids and cholesterol.

Age-group	FFA mmol/l	Glycerides mmol/l	p	Phospho-lipids mg/100 ml	p	Cholesterol mg/100 ml	p	Ketone bodies mmol/l	Glucose mg/100 ml
Cord blood	0.38 ± 0.03 (22)	0.41 ± 0.04 (22)	**	96 ± 4 (22)		65 ± 3 (22)		0.27 ± 0.04 (24)	77 ± 3 (23)
0–24 hours	1.25 ± 0.11 (9)	0.72 ± 0.12 (12)		115 ± 9 (12)		85 ± 8 (12)	—	0.43 ± 0.10 (11)	43 ± 4 (12)
2–3 days	1.0 ± 0.10 (10)	1.18 ± 0.10 (13)	—	156 ± 8 (13)	—	103 ± 7 (13)		0.90 ± 0.15 (7)	44 ± 4 (13)
4–5 days	0.94 ± 0.06 (10)	1.18 ± 0.09 (10)	—	163 ± 7 (10)	—	126 ± 9 (10)	–	0.75 ± 0.09 (11)	49 ± 3 (16)
6–7 days	0.86 ± 0.07 (10)	1.08 ± 0.09 (11)		153 ± 8 (11)		120 ± 10 (11)		0.44 ± 0.06 (12)	46 ± 3 (15)

Chemical Methods

Blood glucose was determined according to the method described by Hultman [17] Plasma FFA were analysed using the method of Dole as modified by Trout [33]. Plasma glycerol was determined with the enzymatic method described by Wieland [36] and ketone bodies were determined as total acetone using the method of Greenberg & Lester in the modification of Werk *et al.* [35]. The re- ... plasma lipids were determined as follows: Glyceride-glycerol according to Carlsson & Wadstrom [7], total cholesterol according to Cramér & Isaksson [9] and phospholipids according to Svanborg & Svennerholm [32]. All analyses were done in duplicate.

The analytical errors for the different methods were: FFA 4.3, ketone bodies 6.4, glycerol 4.4 cholesterol 1 1 phospholipids 2.9 and glyceride-glycerol 2.8 %.

Results

The mean values and standard errors of the mean for FFA, glycerol, ketone bodies and blood sugar are given in Fig 1 In cord blood there are low concentrations of FFA and glycerol. During the first week of life, however there are very high concentrations of FFA glycerol and ketone bodies. The rise of FFA and glycerol is evident within a short time after delivery (Fig 3) The peak values for glycerol, FFA and ketone bodies are reached within the first two days of life. Blood glucose determinations exhibit at this point the lowest values. Highly significant differ ences occur for FFA, glycerol and blood sugar between cord blood and the 5–24 hours age group (see Table 1) With increasing age there is a progressive rise in the blood sugar concentration toward the adult normoglycemic range and a marked decrease of FFA, glycerol and ketone bodies. Further values and some statistical data are given in Table 1

The correlation between FFA and glycerol is highly significant in the following age groups: 5– 4 hours ($r = 0.63$ $n = 24$), 1–2 days ($r = 0.86$, $n = 28$) 2–0 days ($r = 0.84$, $n = _5$), significant in the group

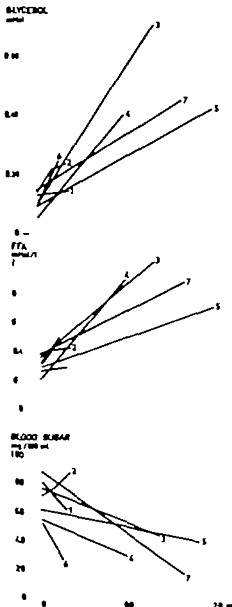


Fig 3 Changes in the concentrations of blood glucose, FFA and glycerol in 7 full term infants during the first two hours of life.

1–6 months ($r = 0\cdot49$ $n = 28$) non-significant in cord blood ($= 0\cdot41$ $n = 23$) and in the age group 1–8 years ($r = 0.50$ $n = 15$) No evidence of an inverse correlation

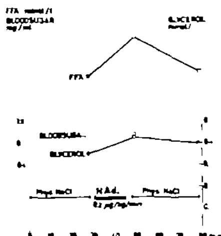


Fig. 4. The effect of intravenous noradrenaline infusion on blood levels of glucose, FFA and glycerol in a 12 hrs old infant born at term. Environmental temperature was kept between 33–33°C.

was found between plasma, FFA and blood sugar in any group.

In Fig 2 the mean levels and the standard errors of the mean for *glycerides, phospholipids, cholesterol, FFA, ketone* bodies and blood sugar are given. The values *for* FFA, ketone bodies and blood

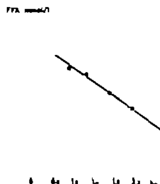


Fig. 5. Mean values for FFA (see Table I) plotted against mean I_g values *obtained from* previous study [13] in different age-groups. 1 – 5 – 24 hrs, 2 – 2 – 9 days, 3 – 1 8 years, 4 – 1 – 6 months. (Statistical analyses have not been done since the data are obtained from two different studies.)

sugar correspond well with those given in Table 1 for the same age groups. During the first days after delivery there is a rise in the levels of glycerides, phospholipids and cholesterol with peak values at 2–3 days and at 4–6 days for the two latter fractions respectively

For glycerides, phospholipids and cholesterol a highly significant difference was found between cord blood and age group 2–3 days but not between the former and age group 0–24 hours (Table 2) No significant correlation occurs between FFA and glycerides in any group

Discussion

This study has confirmed earlier observations of a rapid rise in plasma free fatty acid level in the infant born at term [11] The rise in FFA is accompanied by a simultaneous increase of the glycerol level in plasma, that is correlated highly significantly to that of FFA and indicates an intense lipolysis. This physiological lipid mobilization begins imme- y after birth, reaching the peak nes for FFA and glycerol during the first 24–48 hours of life Despite an increasing supply of exogenous calories the levels of FFA and glycerol remain elevated (and comparable to the levels seen in untreated juvenile diabetics) during the first week of life suggesting a continuous accelerated hydrolysis of stored triglycerides

The enhanced lipolysis during the neonatal period is further evidenced by the pronounced increase in blood ketones This study also shows that there is a highly significant decrease of the FFA, glycerol and ketones after the age of one month

In adults the plasma concentration of FFA is known to be controlled by nutritional, neurogenic and hormonal factors. Lack of utilizable glucose can be one factor that initiates lipolysis in the newborn although the rise in FFA and glycerol seems to start before the glycogen stores are depleted [28]. The release of noradrenaline by thermal stimulation may be an important factor since this sympathetic transmittor is a potent fat mobilizing agent *in vitro* as well as *in vivo* [4, 16]

When newborn human infants are exposed to a cool environment, they respond with an increased oxygen consumption [6] and an increased urinary excretion of noradrenaline and its metabolites [29 31].

Intravenous administration of noradrenaline to newborn infants is accompanied by an increase in the oxygen consumption and followed by thermogenesis [18]. This metabolic response corresponds well with our observation of a rise of FFA and glycerol during infusion of noradrenaline of a similar concentration (see Fig 4).

Another factor of importance may be human growth hormone (HGH) In contrast to the immediate FFA rise augmented by other adipokinetic substances, the plasma FFA response to administrated HGH in adults begins 1–2 hours after the injection and the peak value is reached after 4 hours [3 23 34] In this context it is of special interest that Cornblath *et al.* have demonstrated very high plasma HGH levels in cord blood from full term infants [8]. The mean plasma HGH concentration remained very high during the first 48 hours of life; thereafter there was a gradual fall but still at the age of 8

weeks the concentrations exceeded those measured in adults.

On the other hand newborns have low circulating levels of insulin [2, 30), the only hormone known to decrease the FFA release from adipose tissue, when glucose is abundant. The plasma insulin response to intravenous glucose load is also surprisingly low [2]. These circumstances favour an acceleration of the FFA release

On the basis of different *in vitro* observations, Randle *et al.* have suggested that the FFA and ketone body concentrations, and thus the lipid mobilization, have a regulatory influence on the metabolism of glucose [25]. According to this theory conditions with impaired glucose tolerance such as diabetes mellitus, starvation and obesity are explained by an enhanced lipid mobilization. When the plasma FFA levels are artificially increased by infusion of fat-emulsion, or heparin-injection after a fatty meal, a decrease in the disappearance rate of glucose has been demonstrated [14, 22, 26]. The glucose tolerance is very low during the neonatal period, but increases significantly in infants 1–6 months old, during which age-period the tolerance is even higher than in older children and adults [13]. In this study no statistical correlation was found between fasting blood levels of FFA and glucose. When, however the mean values for glucose tolerance (expressed as the disappearance rate of intravenously injected glucose k_G), found in a previous study in children of corresponding age-groups, are plotted against the corresponding mean FFA values there is a relationship as seen in Fig. 5 This finding of an increasing glucose tolerance with decreasing FFA values could support the hypothesis of Randle *et al.* of a glucose-fatty acid cycle [25] The glucose sparing effect of the increased lipid mobilization during the first week of life is naturally a very useful mechanism, as it guarantees most of the available glucose to the non insulin-dependent tissues (above all the central nervous system).

This study has confirmed earlier observations of very low levels of cholesterol, phospholipids and triglycerides in cord blood [3 12, 20] and of significant rise in all components measured within the first 24 hours of life before the first feeding [12, 24, 27]. The peak value for glycerides is reached at the 2nd–3rd day while cholesterol and phospholipids have their maximum values between the 4th–5th days of life. It seems very probable that the physiologically enhanced FFA release during the neonatal period affects the concentration of blood lipid in a similar way as seen in artificially induced lipid mobilization. Thus the somewhat slower increase in blood glycerides (in comparison to FFA) is in good agreement with the findings produced in animals during prolonged noradrenaline induced fat mobilization [4, 16] From these studies it is apparent that the elevation of liver-glycerides has to persist for a certain period of time before the concentrations of lipoproteins and lipids (except FFA) increase.

Supporting this view is the recent observation of Aherne [1], who demonstrated fatty infiltration in parenchymal and muscular tissues of 62 live born infants who died within the first few days of life. This infiltration was most marked during the first 48 hours.

Dietary factors also must be considered,

although it is established that the initial rise in total lipids is not influenced by the type of dietary fat given from the beginning of life [19]. As in the material of Brody & Carlson [5] there was no correlation between birth weight or sex and the different lipid fractions, but a tendency to a skew distribution of the glycerides was noted.

The glycerol formed during the hydrolysis of glycerides is not reutilized in the adipose tissue, but is either oxidized in, or transformed to glucose, in glycerokinase containing tissues (kidneys, intestinal mucosa, liver) [15, 37]. Glycerol has a very rapid turn-over rate. A sizable fraction of C^{14}-labelled glycerol administered to rats was found incorporated into glucose a few minutes after the injection [21]. If the blood glycerol in human infants has a similar rapid turn-over rate especially during the newborn period, when the concentrations are high, it has to be considered as an important additional source of carbohydrate. If the brain of human infants shows glycerokinase activity one might speculate whether a high up-take of glycerol in the brain could explain why healthy newborns can tolerate low blood sugars.

Summary

Studies of individual blood lipid fractions, glycerol, ketones and blood sugar were carried out in a large group of healthy children ranging in age from full term birth to 8 years, with the object of ascertaining the alterations in these parameters at birth and through the period of postnatal adaptation.

FFA, glycerol and ketone bodies are low at birth then rise rapidly reaching the highest levels within the first 48 hours of life, remaining high during the neonatal period and decrease to the lowest values at 1–6 months. When peak values for FFA and glycerol are reached, blood sugar determinations exhibit the lowest values.

During the first day after delivery before the first feeding glycerides, phospholipids and cholesterol rise. They reach their peak values at 2–3 days and at 4–5 days for the two latter fractions respectively.

There is a highly significant correlation between FFA and glycerol, not however between FFA and glucose during the neonatal period.

The results indicate an intense lipolysis during the neonatal period. The mechanism initiating this lipid mobilization (and redistribution of endogenous stored lipids) is not known. The importance of neurogenic, hormonal and nutritional factors in the regulation of FFA metabolism is discussed. When the values for glucose tolerance (*obtained from a previous study*) are compared to the fasting FFA levels of corresponding age groups, there is a relationship with decreasing FFA values there is an improvement of the carbohydrate tolerance.

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References

1. Aherne, W. Fat infiltration in the tissues of the newborn infant. *Arch Dis Childh, 40* 406, 1965.
2. Baird, J. D. and Farquhar, J. W. Insulin secreting-capacity in newborn infants of normal and diabetic women. *Lancet I* 71, 1962.
3. Basu, A., Passmore, R. and Strong, J. A.: The effects of a human growth hormone preparation on man. *J Physiol, 149* 39 1959.
4. Brodie, B., Maickel, R. and Stern, D. N. *Autonomic nervous system and adipose tissue.* Handbook of Physiology: Section 5. Adipose tissue. Ed. by Renold, A. E. and Cahill, J. G. F. Am. Physiol. Society Washington 1965, p. 583.
5. Brody, S. and Carlson, L. A.: Plasma lipid concentrations in the newborn with special reference to the distribution of the different lipid fractions. *Clin Chim Acta, 7* 694 1962.
6. Brück, K.: General aspects of temperature regulation of small subjects. *The adaptation of the newborn infant to extrauterine life.* Nutricia Symposium. Ed. by Jonxis, J. H. P. Visser, H. K. A. and Troelstra, J. A. Stenfert Kroese, Leiden 1964, p. 228.
7. Carlson, L. A. and Wadström, L.: Determination of glycerides in blood serum. *Clin Chim Acta, 4.* 197 1959.
8. Cornblath, M., Parker, L. M., Reisner, H. S., Forbes, A. E. and Daughaday, W. H.: Secretion and metabolism of growth hormone in premature and full term infants. *J Clin Endocrin Metabol, 25.* 209, 1965.
9. Crawford, K. and Isaksson, B.: An evaluation of the Theorell method for the determination of total serum cholesterol. *Scand J Clin Lab Invest, 11* 212, 1959.
10. Dawkins, M. J. R. and Hull, D.: Brown adipose tissue and non-shivering thermogenesis in newborn animals. *The adaptation of the newborn infant to extrauterine life.* Nutricia Symposium. Ed. by Jonxis, J. H. P. Visser, H. K. A. and Troelstra, J. A. Stenfert Kroese Leiden 1964, p. 269.
11. Van Duyne, C. M. and Havel, R. J. Plasma unesterified fatty acid concentration in fetal and neonatal life. *Proc Soc Exp Biol and Med, 102.* 599, 1959.
12. — The plasma lipids in pregnancy and in the newborn. *Obstet Gynec 3* 326, 1960.
13. von Euler, U. Larsson, Y. and Persson, B. Glucose tolerance in the neonatal period and during the first six months of lif. *Arch Dis Child, 39* 393, 1964.
14. Felber, J. P. and Vannotti, A.: Effects of fat infusion on glucose tolerance and insulin plasma levels. *Med Exp, 10* 153, 1964.
15. Hagemeier, H. A. and Isselbacher, K. J. The metabolism of glycerol by intestinal mucosa. *Biochim Biophys Acta, 73.* 427 1963.
16. Havel, J. R.: *Autonomic nervous system and adipose tissue.* Handbook of Physiology: Adipose tissue. Section 5. Ed. by Renold, A. E. and Cahill, J. G. F. Am. Physiol. Soc., Washington 1965, p. 575.
17. *Hultman, E.: Rapid specific method for* determination of aldosaccharides in body fluids. *Nature, 183* 108, 1959.
18. Karlberg, P. Moore, R. E. and Oliver, T. K.: Thermogenic and cardiovascular responses of the newborn baby to noradrenaline. *Acta Paediat Scand, 54* 225, 1965.
19. Lindquist, B. and Malmcrona, R.: Dietary fat in relation to serum lipids in the normal infant. *Amer J Dis of Child, 99* 39, 1960.
20. Mortimer, J. G. Cord blood lipids of normal infants and infants of diabetic mothers. *Arch Dis Childh, 39* 342, 1964.
21. Nikkilä, E. A. and Ojala, K.: Glycerol as an endogenous precursor of body glucose in the rat. *Ann Med Exp Fenn, 41* 478, 1963.
22. Persson, B., Sterky, G. and Thorell, J. Effect of fat infusion on plasma glucose, FFA, glycerol and insulin levels during intravenous and oral glucose tolerance tests. (To be published in *Proc. 2nd Internat. Symp. on Drugs affecting lipid metabolism,* Milano 1965.)
23. Raben, M. S. and Hollenberg, C. H.: Effect of growth hormone on plasma fatty acids. *J Clin Invest, 38* 484, 1959.
24. Rafstedt, S. Studies on serum lipids and lipoproteins in infancy and childhood. *Acta Paediat Scand Suppl 102* 1955, p. 44
25. Randle, P. J. Garland, P. B., Hales, C. N. and Newsholme, E. A.: The glucose fatty-acid cycle. *Lancet, I* 785, 1963.
26. Schalch, D. S. and Kipnis, D. M.: The impairment of carbohydrate tolerance by elevated plasma free fatty acids. *J Cli Invest, 43* 1283, 1964.
27. Senn, M. J. E. and McNamara, H.: The lipids of the blood plasma in the neonatal period. *Am J Dis Child, 51.* 445, 1937
28. Shelley, H. J. Carbohydrate reserves in the newborn infant. *Brit Med J I* 273, 1964.
29. Stern, L., Lees, M. H. and Leduc, J. Noradrenaline as the mediator of chemical thermogenesis in the newborn infant. *J Pediat 65* 1108, 1964.
30. Steinitz, L., Brazie, J. and O'Brien, D. Serum insulin levels in the normal newborn infant. *J Paediat, 63.* 722, 1963.
31. Schindler, M., Rutenberg, C. R. J. Nor

MANN, J. C. S. and MOORE, R. E.: Environmental temperature and urinary excretion of 3-methoxy-4-hydroxymandelic acid in the newborn. *Lancet, I:* 485, 1961

32. SVANBORG, A. and SVENNERHOLM, L.: Plasma total lipid, cholesterol, triglycerides, phospholipids and free fatty acids in healthy Scandinavian population. *Acta Med Scand, 169* 43, 1961
33. TROUT, D., ESTES, E. H. and FRIEDBERG S. J.: Titration of free fatty acids of plasma. A study of current methods and a new modification. *J Lipid Res, 1* 199 1960.
34. WEIL, R.: Pituitary growth hormone and intermediary metabolism. *Acta Endocrin,* (Kbh) *S ppl. 98,* 1965, p. 12.
35. WERK, E. E., MCPHERSON, H. T., HAMRICK L. W Jr., MYERS, J D and ENGEL, F L.: Studies on ketone metabolism in man. A method for quantitative estimation of splanchnic ketone production. *J Clin Invest, 34* 1256, 1955
36. WIELAND O Eine enzymatische Methode zur Bestimmung von Glycerin. *Biochem Zschr 329* 313, 1957
37. WIELAND O. and SUYTER, M.: Glycerokinase. Isolierung und Eigenschaften des Enzyms. *Biochem Zschr 329:* 320, 1957

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From the Departments of Paediatrics and Clinical Chemistry University Hospital, Uppsala, Sweden

Tryptophan Load Tests and Pyridoxal 5 phosphate Levels in Epileptic Children

I Non progressive Brain Damage and Degenerative Brain Disorders

by BENGT HAGBERG ARNE HAMFELT[1] and OLLE HANSSON

Pyridoxine-responsive fits in early infancy are widely recognized, and have attracted much interest during recent years [1 –, 3 9 11 1]. In some cases, disturbance of vitamin B_6 and tryptophan metabolism has been postulated, and increased urinary excretion of xanthurenic acid has been demonstrated by the tryptophan load test [1 2, 1]. No comparable systematic clinical and metabolic investigations have been made in epileptics of other ages. The aim of the present report is to present such studies on carefully selected clinical series, and to evaluate the biochemical mechanism behind the disturbed tryptophan metabolism by determination of the pyridoxal-5-phosphate level in blood.

In this first part of the study estimations of pyridoxal-5-phosphate in plasma and tryptophan load tests were done in (1) children with epilepsy of obviously exogenous origin and a non-progressive clinical course (2) children with progressive brain disorders with or without epilepsy as a symptom of a severe degenerative process, and, for control (3) children with no disorder of the central nervous system.

Clinical Material

Exogenous epilepsy This group comprises 15 cases with stationary chronic brain syndromes, and with epilepsy as the major clinical problem or at least as one of the main ones. The most important clinical data are given in Table I. Perinatal complications had been present in 11 cases including perinatal asphyxia in 8 and intrauterine asphyxia in 3. Two mothers had had toxicosis of pregnancy and 3 of the children had been premature by weight Postnatal complications had been present in 4 cases, two due to cranial trauma, one to toxoplasmosis encephalopathy and one to a benign intracranial tumour

Case no. 12 was the only one in which both cryptogenic and exogenous factors were probably of importance. On examination 9 children showed mental defects, 3 had cerebral palsy syndromes, and 7 had minor neurological abnormalities. At the time of examination all but 2 were receiving treatment for epilepsy

Progressive brain disorders This is a very heterogeneous group of 13 cases (Table 2) where epilepsy was not present in 4 and only constituted a minor part of a more complicated neurological syndrome in the rest. The group included 3 cases of hereditary ataxia (of whom 1 of the Sjogren Larsson syndrome), 1 of phenylketonuria, 1 of

[1] With the technical assistance of Miss Ulla Netterling.

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32. SANDBORG, A. and SVENNERHOLM, L.: Plasma total lipid, cholesterol, triglycerides, phospholipids and free fatty acids in a healthy Scandinavian population. *Acta Med Scand*, *169* 43, 1961

33. TROUT, D., ESTES, E. H. and FRIEDBERG, S. J.: Titration of free fatty acids of plasma. A study of current methods and a new modification. *J Lipid Res*, *1*. 199 1960.

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35. WERK, E. E., MCPHERSON H. T., HAMRICK L. W J., MYERS, J D and ENGEL, F L.: Studies on ketone metabolism in man. A method for quantitative estimation of splanchnic ketone production. *J Clin Invest*, *34* 1256, 1955.

36. WIELAND, O.: Eine enzymatische Methode zur Bestimmung von Glycerin. *Biochem Zschr* *329* 313, 1957

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TABLE 3. *Children with no disorders of the central nervous system. Controls*

Case, sex, age in years at examination	Clinical diagnosis	XA in urine µmoles/ kg/24 hr	XA per cent of dose tryptophan	P-5-P in plasma ng/ml
1. P P ♂ /12	Social problems	0.4	0.15	6.3
2. L. N ♂ /12	Rumination	2.7	0.57	20.7
3. M. L. ♀, 1 /12	Social problems	1.0	0.37	8.4
4. G. A. ♀, 1 /12	Social problems	1.5	0.21	10.2
5. D M. ♂ 2	Social problems	2.1	0.84	2.6
6. T S. ♂ 3/12	Social problems	3.8	0.78	2.8
7 A. M. A. ♀, 3/12	Social problems	2.8	0.65	12.9
8. G A. ♂ /12	Joint contracture	2.4	0. 0	3.9
9. U W ♂ 2 /12	Developmental anomaly	1.7	0.37	7.2
10. A. E. ♀, 3/12	Developmental anomaly	0.8	0.19	—
11. M. L. M. ♀ 3/12	Healthy	1.1	0.22	4
12. J J ♂ 3/12	Perthes disease	1.8	0.23	12.4
13. J O O. ♂ 4 /12	Healthy	0	0	12.0
14. B. E. ♀, 6	Pruritus	0.3	0.1	20.9
15. T A. ♂ 6	Social problems	0	0	—
16. E. L. ♀, 7	Social problems	3.0	0.62	3.5
17 G M. ♂ 11	Posttraumatic deformities	11.5	2.37	10.3
18. C. J ♀ 14	Social problems	0.01	0.1	3.7

Heller's infantile dementia, 1 of progressive leucoencephalopathy (Mb Schilder 1912), 2 referred to the group of neurolipidoses, 1 of a granulomatous brain degeneration, and finally four of unknown degenerative disorders. One of these four case no. 19 showed atactic paraplegia, oligophrenia, and a disturbance of vitamin B_6–tryptophan metabolism; the main data of this case will be presented in the discussion.

Controls This group comprises eighteen cases (Table 3) with no known disorder of the central nervous system. The patients were 7 months-14 years of age (median 3½ years), and had been referred to the clinic for various reasons. All were normal on neurological examination. In 5 cases electroencephalographic examination was performed and found normal.

Ch mical Methods

1 *Pyridoxal-5-phosphate in plasma*

The pyridoxal 5-phosphate (P-5-P) le l in plasma was determined by a tyrosine decarboxylase method described by Hamfelt [7]. Blood samples were withdrawn with a cannula (polished inner surface) at 8 a.m., two hours before administration of tryptophan. The samples were immediately transported to the laboratory and cooled to +4°C the blood cells separated by centrifugation and the plasma proteins precipitated. The samples were stored frozen and were then analysed for pyridoxal-5-phosphate within 24 hours after collection of the blood. During this time no ppreciable decrease in concentration could be traced. All plasma determinations were performed in duplicate. The standard error of the estimation was about ± 13% calculated from double determinations.

2. *Tryptophan load test*

Each patient was gi en 100 mg/kg body weight of L-tryptophan by mouth ad ministered in a glass of fruit juice t the children and through gastric tube to the infants.

TABLE 4. *The mean value (M), standard error of the mean* (S.E.M.) *and standard deviation* (S.D.) *of plasma pyridoxal-5-phosphate and xanthurenic acid excretion in urine after tryptophan load test found in the three groups investigated*

The significance in the difference between the mean values was calculated according to the Student *t*-test. For I–II the significance for pyridoxal-5-phosphate was $0.40 > P > 0.25$, and for xanthurenic acid $0.40 > P > 0.25$. For (I+II)–III the significance for pyridoxal-5-phosphate was $0.10 > P > 0.05$, and for xanthurenic acid $0.49 > P > 0.48$.

	Pyridoxal-5-phosphate conc. in plasma				Xanthurenic acid excretion in urine				
	No. of cases	*M* ng/ml	±S.E.M.	±S.D.	No. of cases	*M* µmoles/kg/24 hr	±S.E.M.	±S.D.	*M* per cent of dose L-tryptophan
I. Exogenous epilepsy	12	7.2	1.0	3.6	15	1.8	0.4	1.5	0.40
II. Progressive brain disorders	10	8.6	2.2	6.4	11	1.7	0.3	1.0	0.25
III. Controls	16	11.2	2.4	9.6	18	2.2	0.6	2.6	0.47

Three days before the test, the patient was placed on a diet with standardized composition of protein, carbohydrates, and fat, but omitting liver and bananas. Supplementary vitamins had not been given for at least 3 weeks before the load test. The amounts of food consumed were recorded to void extreme variations in food habit during the load test.

The urine was collected in 24-hour specimens before and after tryptophan administration. The total excretion of creatinine in each 24-hour sample was used as a check of correct collection of urines if the difference was more than 25% this was taken to indicate a too-low xanthurenic acid alue owing to careless sampling.

The urinary xanthurenic acid (*XA*) before and after the tryptophan load test was determined as described by Weller & Fichtenbaum [18]. The difference between the xanthurenic acid values in the tw urine samples was considered to represent the xanthurenic acid excreted due to the excess administration of tryptophan. All the determinations were performed in duplicat The standard error of the estimation was ±2.1 %. The amount of xanthurenic acid has been expressed both in µmoles/kg body weight/24 hours and in per cent of the dose of tryptophan given, to allow comparison with data from the literature.

3. *Thin layer chromatography*

For purposes of control, thin layer chromatography on urinary tryptophan metabolites as described by Diamantstein & Erhardt [4] was performed on urine samples from 24 hour specimens before and after tryptophan administration, to confirm the presence of xanthurenic acid in the urine after the load test.

Results

The plasma pyridoxal-5-phosphate level and the result of the tryptophan load test are given for each case in Tables 1–3. The mean values (*M*) the standard errors of the mean (S.E.M.) and the standard deviations (S.D.) are shown in Table 4. The significance of the differences have been statistically calculated by Student *t* test.

A probability of 99 per cent in the difference has been taken as significant. In the control group the mean xanthurenic acid excretion amounted to 2.2 μmoles/kg body weight/24 hours or 0.47% of the dose of L-tryptophan. Xanthurenic acid excretions above 3.8 μmoles/kg body weight/24 hours or 0.8% of the dose of L-tryptophan were considered abnormal.

As can be seen from Table 4 no significant differences between the different groups were found, either in the plasma pyridoxal-5-phosphate levels or in the tryptophan load tests. However it must be pointed out that case no. 10 in the group of progressive brain disorders was excluded from these calculations owing to its extreme deviation from the normal, signifying a special syndrome not representative of the group. It was the only case with abnormal findings in this group: the plasma pyridoxal-5-phosphate concentration was not demonstrable and the tryptophan load test was highly abnormal. Among the patients with exogenous epilepsy only case no. 12 fell outside normal limits on tryptophan load testing. The two abnormal cases will be further discussed below. In the control group case no. 17 showed an abnormal tryptophan load test. The reason for the high excretion of xanthurenic acid in this subject is unknown. In the cases with abnormal tryptophan load tests thin-layer chromatography confirmed qualitatively the pathological excretion of xanthurenic acid.

Discussion

The plasma pyridoxal-5-phosphate levels in all three groups tally closely with those found by Wachstein [13] and Hamfelt [8] in young adults. Compared with earlier findings somewhat higher values are obtained with regard to the xanthurenic acid excretion after tryptophan tolerance testing, both when the excretion is expressed in μmoles/kg body weight/24 hours (mean value 2.2) and compared with the results of Vanella et al. [14] (mean value 0.75) in healthy infants, and when the excretion is expressed as per cent of the dose of tryptophan (0.47 in our cases) and compared with the results of Michael et al. [10] in children (0.31%).

In a preliminary paper [5] we reported 3 children with epilepsy of unknown origin and abnormal tryptophan load tests. The present investigation shows that deviations in the tryptophan metabolism do not usually appear in patients with various types of symptomatic epilepsy. Thus it characterizes neither a group of exogenous epilepsy based on stationary chronic brain syndromes nor a group with different progressive brain disorders. In our series one case in each group showed an abnormal tryptophan load test. These call for more thorough discussion.

Case no. 12 (group of exogenous epilepsy), a girl b. February 2, 1954, the third of five children in a healthy family. Birth had been normal, and the birth weight was 3200 g. She developed normally during her first year. When 1½ years old she started to have repeated fits of dizziness without unconsciousness. At 2½ years she had a period of increased lassitude and general irritability. Two months later her first major fit occurred. A few days later she was brought to the paediatric clinic, febrile and in another major fit. On examination no abnormalities were found except for a diffusely paroxysmal EEG of subcortical type and clear serological evidence of cur

rent toxoplasmosis infection with rising titres. During th next few months she had repeated fits resistant to most anti epileptic treatment but responding best to treatment for toxoplasmosis, i.e. to Daraprim[R] and sulphonamide. This was tried when the titres were found to persist at very high levels for more than a year. However even after her toxoplasmosis had settled down she continued to have bouts of mainly major fits, but was still quit normal in her general development.

It is probable that this patient was suffering from an aetiologically mixed form of epilepsy which was primarily not exogenous but cryptogenic. As will be shown in the second part of these investigations, a large proportion of patients with cryptogenic epilepsy were found to have abnormal tryptophan load tests [6].

Case no. 10 (group f progressive brain disorders) a girl b. March 20, 1957 the eldest of three children in a healthy family. She was born at term, and weighed 3200 g. Motor and mental development was quite normal during her first 6 months, and nearly normal up to one year of age. From that time her intellectual development and emotional rapport deteriorated rapidly. Her speech, consisting of single words disappeared completely. She lost the use of her hands and developed stereotyped movements and her gait became unsteady. No convulsions had occurred. When 3½ years old she was examined at the paediatric clinic, Uppsala. She was found to have a peculiar ppearance stereotyped movements and drooling. She was autistic and severely mentally retarded, without any speech. Her motor development was on a level of pproximately 10–12 months of age. Neurologically she was found to ha ataxia with pronounced intention tremor. She had no signs of spasticity and no hyperkinetic movements. The ophthalmoneurological and otoneurological findings were normal. Electroencephalography showed epileptogenic bursts from both hemispheres. The cerebro-spinal fluid was normal. Brain and rectal biopsy specimens were normal. Numerous laboratory tests were performed, including phenylpyruvic acid tests, sulphatide excretion, amino-acid chromatography and serum lipid studies. All but the serum copper (180–214 µg per 100 ml, normal 80–150), the tryptophan load test and the plasma pyridoxal-5-phosphate level were normal. Therapy with vitamin B_6 (100 mg per day for 11 months) gave no clinical improvement.

This initially healthy girl successively developed an unusual clinical syndrome with severe mental retardation, autism and ataxia. In spite of detailed metabolic investigation involving many different tests, no abnormalities were revealed other than the high serum copper the extremely abnormal tryptophan load test and the lack of demonstrable pyridoxal 5-phosphate in the plasma when vitamin B_6 had not been given. However when given vitamin B_6 (100 mg per day) her pyridoxal-5-phosphate level rose above normal levels, but her tryptophan load test did not become normal until the plasma pyridoxal-5-phosphate level greatly exceeded the normal. This patient is now under continuous observation, and further investigation of her tryptophan metabolism is proceeding. She is obviously suffering from a peculiar metabolic syndrome, and her case is to be published separately in detail at a future dat

Summary

Pyridoxal-5-phosphate in plasma and tryptophan load tests were studied in 15 children with epilepsy due to stationary chronic brain syndromes of an exogenous origin, in 13 children with different progressive brain disorders and in 18 con-

trols. No significant differences were found either in the plasma pyridoxal-5 phosphate levels or in the urinary xanthurenic acid excretion. Two unusual cases are reported in detail, and are briefly discussed.

Acknowledgements

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References

1 Bessey O A., Adams, D J D Bussey D R. and Hansen A. E.: Vitamin B_6 requirements in infants. *Fed Proc, 13*, 451 1954.
2. Cochrane, W A.: The syndrome of infantile spasms and progressive mental deterioration related to aminoacid and pyridoxine metabolism. *Proceedings of IXth International Congress of Pediatrics*, Montreal, 1959, p. 115.
3. Coursin, D B.: Convulsive seizures in infants with pyridoxine-deficient diet *JAMA 154* 406, 1954.
4 Diamantstein T and Ehrhart H.: Dunnschichtchromatographische Trennung einiger Stoffwechselprodukte des Tryptophans. *Hoppe-Seylers Zschr physiol Chem, 326* 131, 1961
5. Hagberg B., Hamfelt A. and Hansson O.: Epileptic children with disturbed tryptophan metabolism treated with vitamin B_6. *Lancet, I* 145, 1964.
6. Hagberg, B., Hamfelt A. and Hansson, O Tryptophan load tests and pyridoxal-5-phosphate levels in epileptic children. II. Cryptogenic epilepsy *Acta Paediat Scand 55* 371 1966.
7 Hamfelt A.: A method of determining pyridoxal phosphate in blood by decarboxylation of L-tyrosine-^{14}C(U). *Clin Chim Acta, 7* 746, 1962.
8. — Age variation of vitamin B_6 metabolism in man. *Clin Chim Acta, 10* 48, 1964.
9. Hunt A. D Stokes, J., McCrory W W and Stroud H. H.: Pyridoxine dependency: Report of a case of intractable convulsions in an infant controlled by pyridoxine. *Pediatrics, 13* 140, 1954.
10. Michael, A. F Drummond, K. N., Doeden D Anderson, J A. and Good, R. A.: Tryptophan metabolism in man. *J Clin Invest, 43*, 1730, 1964.
11. Molony C. J and Parmelee, A. H.: Convulsions in young infants as a result of pyridoxine (vitamin B_6) deficiency *JAMA 154*. 405, 1954.
12. Snyderman S E., Carretero, R. and Holt L. E. Pyridoxine deficiency in the human being. *Fed Proc, 9* 371 1950
13. Wachstein M., Kellner, J D. and Ortiz, J M.: Pyridoxal phosphate in plasma and leucocytes in patients with leukemia and other diseases. *Proc Soc Exp Biol Med 103* 343, 1960.
14. Vasella, F Hellström, B and Wengle, B.: Urinary excretion of tryptophan metabolites in the healthy infant. *Pediatrics, 30* 585, 1962.
15. Weller, H. and Fichtenbaum M.: Der Tryptophanbelastungstest als Nachweis eines Vitamin B_6 Mangels bei Arteriosclerose. *Klin Wschr 39* 1275, 1961

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Tryptophan Load Tests and Pyridoxal 5 phosphate Levels in Epileptic Children

II Cryptogenic Epilepsy

by BENGT HAGBERG ARNE HAMFELT[1] and OLLE HANSSON

In a previous communication [23] the cases of three children with epilepsy of unknown origin and disturbed tryptophan metabolism were reported. Despite normal plasma pyridoxal-5-phosphate values, the urinary excretion of xanthurenic acid after tryptophan load tests was abnormally high. The addition of pyridoxine restored the xanthurenic acid excretion to normal in the presence of a raised plasma pyridoxal-5-phosphate level. This suggested that it is necessary in such cases to raise the plasma pyridoxal-5-phosphate if enzyme reactions dependent on this coenzyme are to function normally

The present paper contains a more systematic, comparative study using the same methods but on a larger clinical series of selected cases of epilepsy of unknown origin. Furthermore, the results will be compared with the findings in groups of cases representing different sorts of symptomatic epilepsy and studied in detail in Part I of this investigation [4].

Clinical Material

The series comprises 43 cases of cryptogenic epilepsy with an overrepresentation of severe cases, many resistant to antiepileptic therapy and many with developing mental and/or minor neurological defects. The main data of each case are collected in Table 1. There were 19 boys and 24 girls. The ages at examination varied from 5 months to 14½ years. All had had normal births. Three had a birth weight of less than 2500 g, one of them being a twin. The neonatal period was reported as normal in all. Apart from the actual disease, no postnatal cerebral complications were known to have occurred in any of the children. Epilepsy was present in the family in 12 cases (Table 2). Three of the children are siblings from one family (cases no. 13, 18, and 33) The age of onset of the fits in each case ppears in Table 1 and a summary of all data is found in Table 3. Of the 10 patients in whom symptoms started before 1 year of age, one was 3 weeks old, 4 were aged 2–6 months, and the rest 6–12 months at the time of onset. The distribution of the various types of fits and the main combinations of them are given in Table 4. No less than 30 patients had psychomotor or minor motor fits combined with other types of fits at one and the same time. Mental defects, most of them slight, were common. Mental stagnation, regression, or severe deterioration was obvious in a total of 24 cases (Table 5). Hyperactivity syndromes were found in 6 children. Irritability and aggressiveness or indolence and inactivity

[1] With the technical assistance of Miss Ulla Netterdag.

TABLE 1 *Children with cryptogenic epilepsy*

Case sex, age in years at exam.	Onset of fits. Age in years	Type of fits	Mental state	Therapy at examination	I-5-P in plasma ng/ml	XA in urine µmoles/kg/24 h	XA per cent of dose tryptophan	Result of tryptophan load test	Response to pyridoxine therapy
1. K. N ♂ /12	/12	Minor motor	Normal	None	31	6.2	1.29	Abnormal	Not tried
2. M. L. ♀ /12		Minor motor	Mental stagnation	Phenobarbital	45	2.1	0.44	Normal	Not tried
3. M. O ♂ 10/12	/12	Grand mal	Normal	None	—	0.8	0.18	Normal	Not tried
4. C. E. ♀ 1 /12	/12	Minor motor Grand mal	Hyperactivity Mental stagnation	Phenobarbital	9 9	0	0	Normal	None
5. E. F ♂ 1 /12	1 /12	Grand mal (single)	Normal	None	7.2	2.9	0.60	Normal	Not tried
6. P. B. ♂ 2	1 /12	Grand mal	Normal	None	14.9	4.1	0.93	Abnormal	Not tried
7. R. M. W ♀ 2/12	1 /12	Minor motor	Mental stagnation	None	6.2	6.6	1.65	Abnormal	No fits, mental progress
8. K. N ♂ 4	4	Grand mal	Mental stagnation	Phenantoin	14 1	2.6	0.52	Normal	None
9. L. O U ♂ 4	3/12	Petit mal	Normal	None	7 6	5.7	1 17	Abnormal	None
10. R. A. ♂ 4	3	Minor motor Grand mal	Slight mental retardation	Ethosuximid Gemonil Acetazolamide	2.8	2.4	0 49	Normal	None
11. S. B. ♂ 4	/12	Grand mal	Slight mental stagnation	Mysoline Phenantoin	4.4	12.2	2.65	Abnormal	Not tried
12. A. S. ♀ 4 /12	4	Minor motor Petit mal Grand mal	Hyperactivity	Mesantoin Phenobarbital	4.2	0.1	0.01	Normal	None
13. L. K. ♂ 4 /12	3	Grand mal Minor motor	Hyperactivity Slight mental stagnation	Gemonil Mysoline	2.6	6.7	1.38	Abnormal	None
14. C W ♀ 5	/12	Minor motor Grand mal	Mental retardation	Methylphenobarbital Mysoline	10 4	0.7	0.14	Normal	None
15. J M. ♂ 6	5	Grand mal	Hyperactivity	Phenantoin	2.4	3.8	0 78	Abnormal	Good
16. I L.	/	Psychomotor	Normal	Phenantoin	5.7	9.3	1 92	Abnormal	None

17. K. M. ♂ 7	4	Minor motor Grand mal	Normal	Paramethadione Phenantoin	4.1	2.3	0.4	Normal	Not tried
18. G. K. ♀ 7	3	Grand mal Minor motor	Hyperactivity Slight mental stagnation	Gemonite Mysoline	9.0	7.1	1.43	Abnormal	None
19. I. B. ♀ 7⁸/₁₂	7	Petit mal	Normal	None	7.5	1.4	0.30	Normal	None
20. J. K. ♂ 8	1 /₁₂	Grand mal Psychomotor	Mental retardation	Mysoline	9.2	2.7	0.46	Normal	Not tried
21. M. J. 8	6⁸/₁₂	Grand mal Petit mal Minor motor	Slight mental stagnation	Ethosuccinimide, Tridione Mysoline	6.6	12.9	2.52	Abnormal	Deterioration
22. A. C. S. ♀ 8	4⁸/₁₂	Grand mal Psychomotor	Behaviour deviations	Phenantoin	10.1	8.4	1.06	Abnormal	None
23. M. H. ♂ 8	1	Petit mal	Mental stagnation	Tridione	9.2	1.4	0.34	Normal	Not tried
24. A. J. ♀ 8⁸/₁₂	3⁸/₁₂	Grand mal Psychomotor	Mental retardation	Ethosuccinimide Phenantoin Phenobarbital	5.5	8.4	1.57	Abnormal	Mental improvement
25. B. J. ♂ 8⁸/₁₂	/₁₂	Grand mal	Mental retardation	Mesantoin Gemonite	8.1	0.3	0.08	Normal	None
26. L. J. ♂ 9	4	Psychomotor	Mental retardation	Tegretol Phenobarbital	11.0	0.9	0.20	Normal	Not tried
27. P. W. ♂ 9	3	Grand mal Psychomotor	Slight mental stagnation	Acetazolamide Phenobarbital Ethosuccinimide Mesantoin	8.0	11.3	2.22	Abnormal	Less fits mental progress
28. L. F. 9⁴/₁₂	6	Grand mal Petit mal Minor motor	Normal	Acetazolamide Ethosuccinimide Phenobarbital	9.5	0.7	0.16	Normal	Not tried
29. M. L. R. ♀ 9	6	Psychomotor Minor motor	Slight mental stagnation	Mesantoin Tridione Methylphenobarbital	12.1	9.0	1.80	Abnormal	None
30. K. N. ♂ 11	3	Grand mal Minor motor	Mental retardation	Phenobarbital Ethosuccinimide Milontine	4.2	6.7	1.43	Abnormal	None
31. R. J. ♀ 11	11	Grand mal	Mental retardation	Phenantoin	—	1.6	0.33	Normal	Not tried
32. M. L. T. 11 /₁₂	/₁₂	Psychomotor	Normal	Phenantoin	2.6	5.8	1.16	Abnormal	Less fits
33. R. K. ♂ 12	4	Grand mal Minor motor	Hyperactivity Slight mental retardation	Gemonite Mysoline	2.5	13.0	2.04	Abnormal	Not tried
34. B. L. 12	8	Petit mal Grand mal	Normal	None	4.5	8.4	1.12	Abnormal	None

Table 1 (continued)

Case, sex, age in years at xam.	Onset of fits. Age in years	Type of f ts	Mental stat	Therapy t examination	P-5 P in plasma ng/ml	XA in urine µmoles/kg/24 hr	XA per cent of dose trypto-phan	Result of tryptophan load test	Response to pyrid xine therapy
35. A. H. ♀ 13[9]/12	4	Psychomotor Grand mal	Mental stagnation	Phenantoin Gemonite	—	5.5	1 11	Abnormal	N fits, mental progress
36. I. A. ♀ 14	13	Psychomotor	Normal	None	19.3	4.5	0.90	Abnormal	Not tried
37 A. M. L. ♀ 14	11	Psychomotor Petit mal Grand mal	Normal	Ethosuccinimide Phenantoin + Tegretol Phenobarbital	7 3	4.9	1 00	Abnormal	Not tried
38. B. E. ♀ 14	13	Psychomotor Grand mal	Mental stagnation	Phenantoin Mysoline	4.2	16.5	3.43	Abnormal	Less fits, mental progress
39 K. W ♀ 14	8	Psychomotor Petit mal Grand mal	Normal	Ethosuccinimide Mesantoine Methylphenobarbital	4.2	7.5	1.54	Abnormal	Excellent
40 Y J ♀ 14 /	11 /12	Psychomotor Grand mal	Normal	Mysoline Phenantoin Phenobarbital	5.7	4 4	0.90	Abnormal	None
41 S. M ♀ 14 /12	13	Psychomotor Grand mal	Normal	Mysoline	3.5	12.3	2.54	Abnormal	Excellent
42. S. S. ♀ 15	6	Grand mal	Mental retardation	Gemonite	4.2	1 3	0.26	Normal	Not tried
43. J O E. ♂ 18	/12	Psychomotor Grand mal	Normal	None	—	11 6	2.39	Abnormal	N t tried

were also found in not a few of the children. Disorientation combined with mental regression was present in case no. 24 (case no. 2 in our preliminary communication [22]). Abnormal neurological findings were elicited in 9 cases; in all of them the changes were minor. All 9 showed disturbance of coordination; in 2 of them this was probably secondary to the drug regimen. In a further 2 of the 9 minor hemiparetic symptoms developed during the course of the illness. The electroencephalogram was examined in all cases, and was found to be normal in only 3. A large variation of pathological patterns was met with; 9 patients showed slow irregular spike-wave rhythms. Pneumoencephalography was performed in 14 cases, 6 of which showed slight central or cortical atrophy. The blood glucose, calcium, phosphorus, and alkaline phosphatase were regularly determined, and these showed normal values in all but case no. 42. This patient developed convulsions at 3 weeks of age, and was later found to have a hypoglycaemic syndrome. At the time of examination 33 of the patients were receiving antiepileptic drug therapy and 10 were receiving no drugs (Table 1). Up t time of writing, pyridoxine given by mouth in doses of 160 mg divided into two doses per day has been tried long enough for evaluation in 26 cases.

Chemical Methods

The pyridoxal-5-phosphate (P-5-P) level in plasma was determined by Hamfelt's method [25]. In the tryptophan load test the child was given 100 mg per kg body weight of L-tryptophan by mouth. The urine was collected in 24-hour specimens before and after tryptophan administration. The xanthurenic acid (X.A.) content of the urine was determined by the method of Weller & Fichtenbaum [46]. Thin-layer chromatography on tryptophan metabolites in the urine was performed as described by Dalgliesh & Erbert [10]. For details concerning the methods, see Part I of this investigation [24].

On the basis of results of an earlier control study [24] the following values have been taken as marginal levels. For pyridoxal-5-phosphate, plasma levels less than 2.5 ng per ml were considered abnormal; for tryptophan load tests urinary xanthurenic acid excretion of more than 3.8 μmoles per kg body weight per 24 hours or 0.78% of the dose L-tryptophan given to the patient were taken as abnormal.

Results

All plasma pyridoxal-5-phosphate levels and all urinary excretions of xanthurenic acid on tryptophan load testing are shown in Table 1. For purposes of statistical calculation, the figures are summarized in Table 6 and compared with the values found in groups of patients with exogenous epilepsy, progressive brain disorders, and controls from Part I [24] of this study. The significance of the differences between the four groups have been calculated by Student's *t*-test. A probability of 99 per cent in the differences is considered significant.

It appears from Fig. 1 and Table 6 that *no significant difference in the pyridoxal-5-phosphate values exists between the four groups.* A significantly raised excretion of xanthurenic acid on tryptophan loading was found in the group of cases with cryptogenic epilepsy as compared to the other groups (Fig.   and Table 6). Of the 43 patients within this group no less than 26 had xanthurenic acid excretions exceeding the accepted upper limit. Of these 43 patients 39 were examined for pyridoxal-5-phosphate concentration in plasma and found normal.

The figures for the limits of pyridoxal-5-phosphate and xanthurenic acid given in our preliminary communication [22] were different, as we had no control series in children at the time.

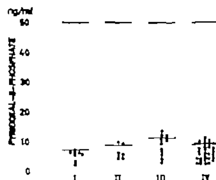


Fig. 1 The distribution of the pyridoxal-5-phosphate concentrations in plasma in the four groups in estigated I, exogenous epilepsy; II, progressive brain disorders; III, controls; IV cryptogenic epilepsy Horizontal line = mean alue (M).

In all cases showing an abnormal tryptophan load test, thin-layer chromatography confirmed qualitatively the existence of xanthurenic acid in the urine Besides the xanthurenic acid a typical pattern of other tryptophan metabolites dominated by kynurenic acid, kynurenine, and 3-hydroxykynurenine was always found in these cases.



Fig. 2. The distribution of the xanthurenic acid excretion in urine in the four groups investigated. (The same abbreviations as in Fig 1)

In 8 cases examined after treatment for a few days with varying amounts of pyridoxine (60–160 mg), the xanthurenic acid excretion after tryptophan load was found to be reduced to normal values while the pyridoxal-5-phosphate level in plasma was increased to 5–10 times the normal.

In 4 of these patients (cases nos. 7 4 27 and 38) attempts were made to titrate the plasma level at which the tryptophan load test became normal (Fig 3)

Tryptophan load tests together with determinations of the pyridoxal-5-phosphate level in plasma were performed at different intervals after the discontinuation of treatment with excess doses of pyridoxine. The results are shown in Fig 3 As can be seen, an abnormal test was obtained even when the plasma levels were still very high thus, the tryptophan load test was considered to have become normal only when pyridoxal-5-phosphate level exceeded the normal

Clinical data are arranged in Tables –5 in accordance with the result of the load test, in an attempt to discover characteristic symptoms and signs to distinguish the patients with abnormal tryptophan load tests from the others. However family history (Table 2) age of onset (Table 3), type of fits (Table 4), and the presence of mental abnormalities (Table 5) were found to be of no help in the clinical selection of abnormal cases. Nor were the EEG changes found to be of any value for this purpose as a wide variation of abnormal patterns were associated both with normal and abnormal tryptophan loads.

Pyridoxine[1] treatment (160 mg per

[1] Hexapyral® kindly supplied by AB Ferrosan, Malmö.

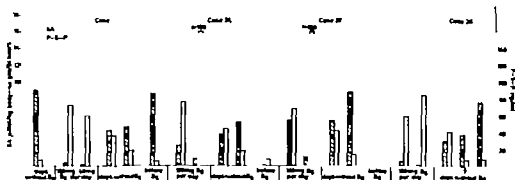


Fig. 2. Comparison between the result of the tryptophan load test and the level of pyridoxal phosphate in plasma in four cases with cryptogenic epilepsy and abnormal tryptophan load test

day) was tried in 26 cases. The results are given in Table 1 Improvement was noted in 9 cases, all of which belonged to the group with abnormal tryptophan load tests. Three children (cases nos. 35 39 and 41) showed immediate and considerable improvement, and two of them were still free from convulsions after one year and six months respectively The third (no. 41), which was a severe case had only two fits during the year after starting vitamin B_6 therapy but immediately suffered 8 during three weeks when pyridoxine was temporarily discontinued. In the rest of the 9 cases only partial relief from the fits could be obtained, but in some of them the antiepileptic drug regimen could be diminished.

Improvement in the mental state occurred in 5 patients (cases nos. 7 15 24, 27 and 38), who had previously stagnated or markedly regressed. This was especially obvious in our first and second cases in the preliminary report [23], where rapid mental deterioration and disorientation improved dramatically (cases nos. 7 and 24) In another group of 10 cases (Table 1) with abnormal tryptophan load tests, vitamin B_6 therapy produced no improvement On the contrary one patient (case no 1) showed an unusually large number of fits during two different periods of vitamin B therapy in another child the epileptogenic EEG changes were accentuated despite clinical improvement None of 7 patients with a normal trypto-

TABLE 2. *Family history of epilepsy related to result of tryptophan load test*

	Normal tryptophan tolerance test	Abnormal tryptophan tolerance test	Total no. cases
Epilepsy in the family	5	7	12
No epilepsy in the family	12	19	31

TABLE 3 *Age of onset of epileptic fits related to result of tryptophan load test.*

Onset of fits Age in years	Normal tryptophan tolerance test	Abnormal tryptophan tolerance test	Total no. cases
1	5	5	10
1 3	3	2	5
3–6	5	10	15
> 6	4	9	13

Table 4. *Type of epileptic fits related to age of patient and result of tryptophan load test*

Figures in brackets refer to cases with abnormal tryptophan load test.

Age in years at exam.	Major motor alone	Petit mal with or without major motor	Psychomotor with or without major motor and/or minor motor	Minor motor with without major motor and/or petit mal	Total no. cases
½–3 years	3 (1)	0	0	3 (1)	6 (2)
3–6 years	2 (1)	1 (1)	0	5 (2)	8 (4)
6 years	4 (2)	3 (1)	16 (13)	6 (4)	29 (20)
Total no. cases	9 (4)	4 (2)	16 (13)	14 (7)	43 (26)

Table 5 *Presence of mental abnormalities related to tryptophan load test*

	Normal tryptophan load test	Abnormal tryptophan load test	Total no. cases
Normal mental development	6	13	19
Mental stagnation	4	10	14
Moderate mental retardation (IQ about 50–60)	5	3	8
Severe mental retardation (IQ less than 50)	2	0	2
Total no. cases mental defects	11	13	

Table 6. *The mean value (M) standard error of the mean* (s.e.m.) *and standard* [illegible]on (s.d.) *for pyridoxal-5-phosphate concentration in plasma and xanthurenic acid excretion in urine after tryptophan load test found in the four groups investigated*

N significant difference was found between the mean values of the pyridoxal-5-phosphate concentrations. A significant difference ($0.0005 > P$ according to the Student's t-test) was found bet een group IV and groups I III. (The same abbreviations as in Fig. 1)

	Pyridoxal-5-phosphat conc. in plasma				Xanthurenic acid xcretion in urine				
	No. of cases	M ng/ml	±s.e.m.	±s.d.	N of cases	M μmoles/kg/24 hr	±s.e.m.	±s.d.	M per cent of dose L-tryptophan
I. Exogenous epilepsy	12	7.2	1.04	3.6	1	1.5	0.39	1.5	0.40
II. Progressive brain disorders	10	8.6	1.03	4.4	11	1.7	0.33	1.0	0.35
III. Controls	16	11.2	2.48	9.9	18	2.2	0.61	2.6	0.47
IV. Cryptogenic epilepsy	20	9.1	1.11	6.9	43	5.3	0.65	4.3	1.10

phan load test showed any response to pyridoxine treatment In the rest of the cases pyridoxine therapy was either not tried or could not be assessed owing to the shortness of the observation period.

Discussion

The tryptophan load test has been widely used for revealing vitamin B_6 deficiency The test is based on the observation of Lepkovsky [19 37 38] in 194_ that pyridoxine-deficient dogs and rats have increased urinary excretion of xanthurenic acid, which is further raised by tryptophan administration and restored to normal by supplementation with vitamin B_6. Since then a large number of publications on the tryptophan load test in various disorders have appeared [1 5 11 13, 14, 22, 32, 34–36 43, 44 47–53 60–63 66]

In paediatrics the tryptophan load test has gained a special place since a relationship was shown between vitamin B_6 and the appearance of fits in infancy [17 56 57] In the "Vitamin B_6 Deficiency Syndrome" [2, 10 12, 45, 55 64] and sometimes in the "Infantile Spasms Syndrome" [4, 7 9 27 33 53] abnormal tryptophan load tests are well documented, and the test has been found to be normal in the "Vitamin B_6 Dependency Syndrome" [8, 15, 21 30 40–4_, 46, 54, 58, 64 65, 67]

In older children with epileptic disorders only single cases investigated with the tryptophan load test have been published [6, 39]. An increasing number of cases with abnormal tryptophan metabolism have recently been reported [23, 28, 29 55 59] however

In cases without obvious dietary vitamin B_6 deficiency there have been difficulties in the evaluation of an abnormal tryptophan load test. In the "Vitamin B_6 Deficiency Syndrome it has been stated that an increased daily intake of vitamin B_6 is required to prevent an abnormal tryptophan metabolism [2, 10 31]. It has not been shown whether a real decrease in the pyridoxal-5-phosphate level has been present in these cases. This is due to the fact that a simple and sensitive method has not previously been available.

In the present study abnormal tryptophan load tests have been shown in not less than two-thirds of children with cryptogenic epilepsy despite normal plasma pyridoxal-5-phosphate levels. Thus a vitamin B_6 deficiency does not seem to be responsible for the abnormal tryptophan load test. Of course the possibility remains of a decreased intracellular pyridoxal-5-phosphate concentration. A certain correlation seems to exist between the pyridoxal-5-phosphate concentration in plasma and that in erythrocytes and leucocytes [26], however Normal pyridoxal-5-phosphate values in these cells were found in two patients (cases nos. 29 and 38) with abnormal tryptophan load tests.

TABLE 7 *Antiepileptic regimen related to result of tryptophan load test*

Antiepileptic regimen	Normal tryptophan load test	Abnormal tryptophan load test	Total no. cases
on phenantoin	12	14	26
without phenantoin	19	13	32
on barbiturates	18	11	29
without barbiturates	13	16	29

It might be argued that the treatment with antiepileptic drugs interferes with the results of the tryptophan load test [48] To judge from the data in Table 7 this seems to be an unlikely explanation for the high frequency of abnormal tests in our clinical series. Medication both with phenantoin and/or barbiturates was at least as often combined with a normal tryptophan load test as with an abnormal one, judging our three different clinical groups (exogenous epilepsy cryptogenic epilepsy with normal tryptophan metabolism, and with abnormal) together Seven cases of cryptogenic epilepsy with abnormal tryptophan load tests had never received any antiepileptic drugs. The mean value for the xanthurenic acid excretion in these cases was 5 1 μmoles/kg body weight/24 hours (S.D ±5.8) and the average pyridoxal-5-phosphate for 6 of them was 12.7 ng/ml (S.D ±15.8).

The increased excretion of xanthurenic acid in patients showing an abnormal tryptophan load test is supposed to be due to lowered activity in the enzyme reaction between 3-hydroxykynurenine and 3-hydroxyanthranilic acid. In this reaction pyridoxal-5-phosphate is the coenzyme to kynureninase Possible aetiological explanations for an abnormal tryptophan load test are as follows.

1 Dietary deficiency and/or defective resorption of vitamin B
2 Abnormal excretion and/or degradation of vitamin B_6 and/or pyridoxal-5-phosphate
3 Insufficient production of pyridoxal-5-phosphate
4. Disturbance in the pyridoxal-5-phosphate–apoenzyme complex.
5 Disturbance in the pyridoxal-5-phosphate–apoenzyme–substrate complex.

The normal plasma pyridoxal-5-phosphate values in our cases constitute strong evidence against the existence of a vitamin B_6 deficiency and are not in keeping with a disturbance in the phosphorylation of pyridoxine to pyridoxal-5-phosphate On the other hand, as is shown in Fig 3 raised plasma levels of pyridoxal-5-phosphate are required in order to get a normal tryptophan load test. This indicates a dependency on a raised pyridoxal-5-phosphate concentration in the organism, possibly due to a decreased affinity of pyridoxal-5-phosphate to kynureninase Other pyridoxal-5-phosphate-dependent enzymes not involved in tryptophan degradation might also be abnormal, but have not been examined in this investigation.

From the clinical point of view attempts were made to predict the outcome of the tryptophan load test with the aid of the family history the age of onset, the type of fits, mental abnormalities, and electroencephalographic changes. However on the basis of these criteria it proved impossible to pick out the cases with abnormal tryptophan metabolism from the normal ones.

Only a few publications concerning pyridoxine therapy in epilepsy starting after early infancy have appeared In the first of these, Fox & Tullidge [20] reported the daily administration of up to 100 mg of pyridoxine to eight epileptic boys aged 14–15 years; the treatment was continued for four weeks without improvement Livingstone *et al* [39] presented another series of 31 children ranging

in age from 6 months to 1.. years with epilepsy of various types; these children were also given doses of 100 mg daily for at least one month and no evidence of improvement was found. Ernsting & Ferwerda [18], however reported 14 patients, 2 to 17 years old, with petit mal, of whom 5 were completely cured and 3 showed improvement on pyridoxine in doses of 60–120 mg daily. In none of these clinical series were tryptophan tolerance tests systematically carried out.

During the last years improvement in epileptic children with abnormal tryptophan metabolism on pyridoxine therapy has been reported by Scriver [55], Hagberg *et al.* [23], and Swaiman [59], whereas Hottinger *et al.* [28, 29] have found this therapy of no value.

In our cases the results of treatment with vitamin B_6 were contradictory. A few cases with an excellent response and some with a more modest one contrast against cases where no effect or even deterioration was observed. A somewhat better therapeutic result might perhaps have been gained if so many of the epilepsies had not already become firmly established before pyridoxine treatment was started. Even if vitamin B had been started after the first fit, however it seems unlikely that many of our cases would have responded with full relief. A more likely purely clinical interpretation is the possibility mentioned above, that different biochemical abnormalities are at work in a group of cases with cryptogenic epilepsy. This theory might be in keeping with a good clinical effect in some cases, but only with normalization of the tryptophan load test in others.

General Conclusions

Disturbance in the metabolism of tryptophan dependent on pyridoxal-5-phosphate seems to be common in children with cryptogenic epilepsy. At present the tryptophan load test is the only means of detecting such disturbances, which constitute an indication for a therapeutic trial with pyridoxine. The optimum therapeutic dose of pyridoxine has not been determined in the present study.

Whether or not in cases showing an abnormal tryptophan load test early treatment with pyridoxine will prevent permanent cerebral damage and thus resistance to adequate therapy is an important question for further investigation.

Summary

Studies were performed on the plasma pyridoxal-5-phosphate level and the urinary xanthurenic acid excretion after a tryptophan load in 43 children with cryptogenic epilepsy. No significant difference in the pyridoxal-5-phosphate values was obtained in comparison with a control group. In contrast, significantly raised excretion of xanthurenic acid characterized the cryptogenic group: Of the 43 patients no fewer than 26 showed an excretion exceeding the accepted upper limit. Treatment with pyridoxine restored the tryptophan load to normal in the presence of a raised plasma pyridoxal-5-phosphate concentration.

It proved impossible clinically to differentiate patients with an abnormal tryptophan load test. Thus family history, age of onset, type of fits, mental symptoms, and the type of EEG-changes were of no help for distinguishing them from

other cases in a group of epileptics. The type of antiepileptic drug treatment did not seem to influence the result of the tryptophan load test, and even patients receiving no drugs responded abnormally to tryptophan loading.

Pyridoxine treatment (160 mg per day) was given in 26 patients, 19 of whom showed an abnormal tryptophan load test. In only 9 of these 19 was improvement noted. None of 7 cases showing a normal tryptophan load test responded to pyridoxine treatment.

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References

1. Benassi, C. A., Allegri, G., Benassi, P. and Rabassini, A.: Tryptophan metabolism in special pairs of twins. *Clin Chim Acta, 9*: 101, 1964.
2. Bessey, O. A., Adam, D. J. D., Bussey, D. R. and Hansen, A. E.: Vitamin B_6 requirements in infants. *Fed Proc 13*: 451, 1954.
3. Bessey, O. A., Adam, D. J. D. and Hansen, A. E.: Intake of vitamin B_6 and infantile convulsions: A first approximation of requirements of pyridoxine in infants. *Pediatrics 20*: 33, 1957.
4. Bower, B. D.: The tryptophan load test in the syndrome of infantile spasms with oligophrenia. *Proc Roy Soc Med, 54*: 540, 1961.
5. Boyland, E. and Williams, D. C.: The metabolism of tryptophan: 2. The metabolism of tryptophan in patients suffering from cancer of the bladder. *Biochem J 64*: 578, 1956.
6. Calvario, M.: Carenza di piridossina ed epilessia. *Acta vitamin, 1*: 23, 1958.
7. Carrer, P.: Veränderungen des Tryptophanstoffwechsels bei den Blitz-Nick-Salaam-Krämpfen des Kindesalters. *Helv Paediat Acta, 17*: 398, 1962.
8. Christiaens, L., Briet, B. and Debarge, Ph.: Un cas familial de convulsions pyridoxino-sensibles. *Pédiatrie, 17*: 161, 1962.
9. Cochrane, W. A.: The syndrome of infantile spasms and progressive mental deterioration related to amino acid and pyridoxine metabolism. *Proceedings of IXth International Congress of Pediatrics*, Montreal 1959, p. 115.
10. Coursin, D. B.: Convulsive seizures in infants with pyridoxine-deficient diet. *JAMA 154*: 406, 1954.
11. — Present status of vitamin B_6 metabolism. *Amer J Clin Nutr 9*: 304, 1961.
12. — Vitamin B_6 deficiency in infants. A follow-up study. *Amer J Dis Child 90*: 344, 1955.
13. Dahler, R. P.: Untersuchungen über den Tryptophanabbau und den Vitamin B_6-stoffwechsel beim Neugeborenen und beim Säugling. *Ann Paediat, 200*: 346, 1963.
14. Dahler Vollenweider, R. M.: Xanthurensäureausscheidung nach Tryptophanbelastung bei Kindern. *Ann Paediat, 200*: 331, 1963.
15. Dekeyser, V. & Jongbloet, P.: Convulsies in de neonatale periode beinvloedbaar door pyridoxine. *Maandschr Kindergeneesk, 29*: 177, 1961.
16. Diamantstein, E. and Ehrhart, H.: Dünnschichtchromatographische Trennung einiger Stoffwechselprodukte des Tryptophans. *Hoppe-Seylers Ztschr physiol Chem, 326*: 131, 1961.
17. Elliot, J. W.: Occurrence of convulsions in infants as a possible manifestation of deficiency of vitamin B_6. *Report of the Tenth M & R Conf*, Columbus, Ohio 1953, p. 53.
18. Ebbeling, W. and Ferwerda, T. P.: De Behandeling van Epilepsie met Vitamine B_6 (Pyridoxine, Adermine). *Nederl T Geneesk 95*: 3042, 1951.
19. Fouts, P. J. and Lepkovsky, S.: A green pigment-producing compound in urine of pyridoxine-deficient dogs. *Proc Soc Exp Biol Med, 50*: 221, 1942.
20. Fox, J. T. and Tullidge, G. M.: Pyridoxine (Vitamin B_6) in epilepsy. A clinical trial. *Lancet, II*: 345, 1946.
21. Garty, R., Yonis, Z., Braham, J. and Steinitz, K.: Pyridoxine-dependent convulsions in an infant. *Arch Dis Child 37*: 21, 1962.
22. Gehrmann, G.: Das Pyridoxin-Mangelsyndrom beim Menschen. *Ergebn Inn Med Kinderheilk, 19*: 274, 1963.
23. Hagberg, B., Hamfelt, A. and Hansson, O.: Epileptic children with disturbed tryptophan metabolism treated with vitamin B_6. *Lancet, I*: 145, 1964.

24. — Tryptophan load tests and pyridoxal-5-phosphate levels in epileptic children. I. Non-progressive brain damage and degenerative brain disorders. *Acta Paediat Scand* *55*, 363, 1966.
25. HAMFELT, A.: A method of determining pyridoxal phosphate in blood by decarboxylation of L-tyrosine-^{14}C(U). *Clin Chim Acta*, *7* 746, 1962.
26. — Unpublished observations.
27 HELLSTRÖM, B. and VASSELLA, F Tryptophan metabolism in infantile spasms. *Acta Paediat Scand*, *51* 665, 1962.
28. HOTTINGER, A. and BERGER, H.: Vorläufige Bemerkungen zur Ausscheidung von Xanthurensäure und Kynurensäure nach Tryptophanbelastung. *Internat Zschr Vitaminforsch*, *34*, 61, 1964.
29. HOTTINGER, A., BERGER, H. and KRAUTHAMMER, W Klinische Beobachtungen zum Probleme des Vitamin-B$_6$ Metabolismus. *Schweiz Med Wschr* *94* 221 1964.
30. HUNT A. D STOKES, J McCRORY W W and STROUD, H. H.: Pyridoxine dependency: Report of a case of intractable convulsions in an infant controlled by pyridoxine. *Pediatrics*, *13*: 140, 1954.
31. HUNT A. D Abnormally high pyridoxine requirement. Summary of evidence suggesting relation between this finding and clinical pyridoxine deficiency" *Amer J Clin Nutr* *5* 561, 1957
32. JÉROME, H., LEJEUNE, J and TURPIN, R.: Étude de l'excrétion urinaire de certains métabolites du tryptophan chez les enfants mongoliens. *CR Acad Sci* (*Paris*), *251* 474, 1960.
33. JEUNE, M., COTTE, J HERMIER, M., YASSEN, L. and LEBOCHER, MME: L'épreuve de charge au tryptophane comme moyen de détection des apyridoxinoses chez l'enfant. *Pédiatrie*, *14* 853, 1959.
34. KRAFT A.: Familiäre essentielle Tryptophanstoffwechselstörung. *Klin Wschr* *38* 74, 1960.
35. KOMROWER, G. M., WILSON V CLAYTON J B. and WESTALL, R. G Hydroxykynureninuria: A case of abnormal tryptophan metabolism probably due to deficiency of kynureninase. *Arch Dis Childh*, *39* 250, 1964
36. DELANEY P HOOFT C., TIMMERMANS, J and SNOECK, J Biochemical aspects of the Hartnup disease. *Ann Paediat* *202*, 221 1964.
37 LEPKOVSKY S. and NIELSEN, E.: A green pigment-producing compound in urine of pyridoxine-deficient rats. *J Biol Chem*, *144* 135, 1942.
38. LEPKOVSKY S. ROBOZ, E and HAAGEN-SMIT, A. J Xanthurenic acid and its role in tryptophan metabolism of pyridoxine deficient rats. *J Biol Chem* *149* 195, 1943.
39. LIVINGSTON S., HSU J M. and PETERSEN D C.: Ineffectiveness of pyridoxine (vitamin B$_6$) in the treatment of epilepsy *Pediatrics*, *16* 250, 1955.
40. MARIE, J., HENNEQUET A., LYON G DEBRIS, P and LE BALLE, J-C.: La pyridoxino-dépendance, maladie métabolique exprimant par des crises convulsives pyridoxino-sensibles (première observation familiale). *Rev Neurol*, *105* 406, 1961.
41. — Les crises convulsives pyridoxino-sensibles d nouveau-né et du nourrisson. *Ann Pédiat*, *35* 197 1959.
42. — Sur un syndrome convulsif particulier d nouveau-né et du nourisson: "les crises convulsives pyridoxino-dépendantes *Presse Méd*, *67* 566, 1959.
43. MARVER, H. S.: Studies on tryptophan metabolism I. Urinary tryptophan metabolites in hypoplastic anemias and other hematologic disorders. *J Lab Clin Med*, *58* 425 1961
44. McCOY E. E. and CHUNG, S. L: The excretion of tryptophan metabolites following deoxypyridoxine administration in mongoloid and nonmongoloid patients. *J Pediat*, *64* 227 1964.
45. MOLONY C. J and PARMELEE, A. H.: Convulsions in young infants as a result of pyridoxine (vitamin B$_6$) deficiency *JAMA*, *154* 405, 1954
46. NORDIO, S., SEGNI, G and GANDULLIA, E.: "Convulsioni piridossino-dipendenti. Primo contributo della letteratura italiana. *Minerva Pediat* *13*, 925, 1961
47 O'BRIEN D and GROSHEK, A. The abnormality of tryptophane metabolism in children with mongolism. *Arch Dis Child*, *37* 17 1962.
48. O BRIEN D and JENSEN C. B.: Pyridoxine dependency in two mentally retarded subjects *Clin Sci*, *24* 179, 1963.
49 OKA, M. and LEPPÄNEN, V V E.: Metabolism of tryptophan in diabetes mellitus. *Acta Med Scand*, *173*, 361 1963.
50. PIAZZA, M. and TANCREDI, F Tryptophan-nicotinic acid metabolism in subjects recently or long since recovered from viral hepatitis. *Nature*, *197* 902, 1963.
51 PRICE, J M.: Disorders of tryptophan metabolism. *Univ Mich Med Bull*, *24* 461 1958.
52. PRICE, J M., BROWN R. R. and PETERS, H A.: Tryptophan metabolism in porphyria, schizophrenia, and variety of neurologic and psychiatric diseases. *Neurology* *9* 456, 1959.
53. SCHWARZ TIENE, E. and CARLO U P Alterazioni del metabolismo del triptofano nella fenilchetonuria, nell'spasmus, nel mongolismo in alcune cerebropatie infantili. *Minerva Pediat*, *14* 1471 1962.

have been included because only part of the period mentioned above is covered by patients from this Clinic.

The children coming from the Department of Pediatrics have either been in-patients or have been treated in the Out patient Department. The area referring patients to the Clinic has about 200 000 inhabitants and besides the staff of the Clinic but a few pediatricians are working there, which means that it receives a rather unselected group of sick children. The Department of Pediatrics also receives children for special examinations from a region having about 1 mill. inhabitants. Therefore, a minor part of the EEG' are performed on children of special interest from the neurological point of view both with regard to diagnosis and therapy

The above-mentioned EEG material was surveyed by the authors in 1964. All the curves were reexamined and classified according to certain fixed criteria and definitions. Furthermore, we tried to judge the anamnesis with regard to epileptic manifestations without being influenced by the results of the EEG-examination. The relevant information was noted on punch-cards.

In the total material of 746 boys and 604 girls we found 212 boys and 160 girls who were judged as suffering from epilepsy ... the attacks described in the case reports were convincingly epileptic in nature. 194 boys and 190 girls were recorded as doubtful but possible epileptogenic. In this group all patients with for example abdominal pain or headache of paroxysmal character were placed. The remaining group, consisting of 340 boys and 254 girls, were judged as non-epileptic

Methods

Most recordings were performed on a Kaiser 8-channel electroencephalograph but some of them on an Elema Mingograph-EEG type EM 160 In the former case 8 channels were used, in the latter 16 channels. Electrode positioning was in accordance with the 10–20 system, mostly with 21 electrodes, but in some cases with 14 electrodes. In most cases both bipolar and average reference recordings were used.

Resting recording was performed during 20–30 minutes, followed by hyperventilation for 3 minutes if the patients were old enough to cooperate. Most examinations ended with photostimulation. In some cases sleep recording was also obtained, either spontaneous sleep or barbiturate-induced. This is valid for 13 of the 26 patients in the Rolandic spike group.

Definitions

1 "*Epileptogenic*" *activity*. This term is used (without clinical implications) in the reports to the referring physician about spikes (including spike and waves) and sharp waves. Spikes and sharp waves differ from each other only with regard to the duration of the potential. The spike potential has a duration of 1/13 sec or less and the sharp waves of 1/15 sec or more. The amplitude of the potential in question (as compared with the background activity) is however of equal importance: it should stand out quite clearly from the background activity which usually means that the amplitude of the potential ought to be twice or more. Abnormalities which are not "epileptogenic" are frequently referred to as "unspecific"

2. *Focus* This term is used quite restrictively *The abnormality has to be localized to* a restricted cortical area, on one side only Mostly it is limited to two or three adjacent derivations and is seen both in longitudinal and transverse leads. Since the term "focus" among referring physicians may signify a restricted cortical lesion we tend to avoid the term in our concluding remarks to the physician unless the EEG findings are indicative of such a lesion (focal, irregular theta or delta activity).

3. *Background activity*. Normal or abnormal continuous activity in contrast to episodic or "epileptogenic" activity

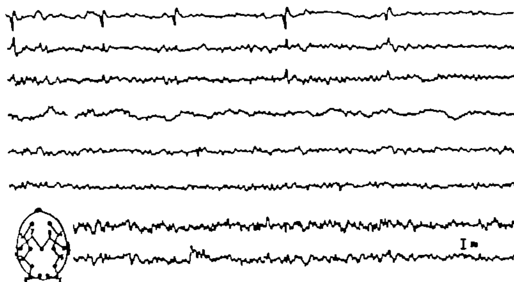


Fig. 1 Bipolar recording, 21 lectrodes, patient awake. Rolandic spikes from left hemisphere. Patient no. 23, Table 1.

Results

The material was divided into the following three groups:

1 *Focal spikes or sharp waves in the central region without disturbed background activity (Rolandic spikes)*

This is the group of "Rolandic spikes" consisting of 26 children. There were 18 boys and 8 girls, age range 1–15 years when the first EEG was recorded. A typical example of this EEG pattern is shown in Fig. 1

Some characteristic features of these Rolandic spikes they are often grouped but may occur single. They may or may not be blocked by eye opening Sometimes, but not always, they disappear during sleep. They are usually strictly unilateral but sometimes smaller synchronous discharges may appear in homologous contralateral areas. If repeated recordings are made with the same patient it may be found that the discharges can temporarily be lost (leaving a perfectly normal EEG) to appear again later in the same manner as before. We have one case in which the discharges were absent at 4 registrations (between 5 and 9 years of age), but reappeared when the patient was 9 years old. In some cases photic stimulation gives rise to bilateral synchronous paroxysmal discharges.

The clinical features of these patients are summarized in Table 1 The upper half of the table show the types of seizures, the lower half additional neurological signs or diagnoses. The following points are worth emphasizing

Twelve of the 26 children had no seizures prior to the investigation. Of these 12 patients four had CP (Cerebral Palsy) syndromes of different types, two had malformations of the brain, (one an

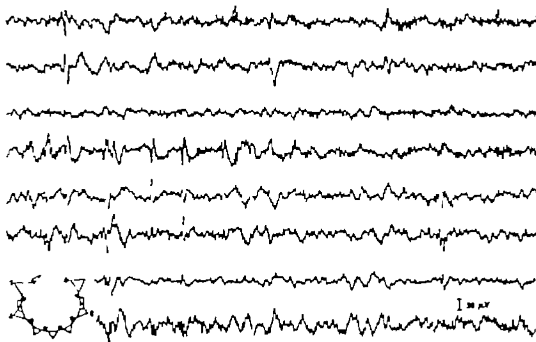


Fig. 2. Bipolar recording, 14 electrodes, patient asleep. Focal spikes from left fronto-temporal region. Patient no. 1 Table 2.

agenesis of the callosal body and one a meningocele in the occipital region) one a tumour in the posterior fossa and five more unspecific paroxysmal symptoms (paroxysmal headache or paroxysmal vertigo) Of the 14 patients with seizures 3 had CP syndromes, while in the other 11 the seizures were the only sign of disease in the central nervous system.

All the 11 patients with seizures but without any other neurological disturbance had either grand mal attacks or asymmetrical motor attacks with or without loss of consciousness. One of these had also psychomotor seizures. No true Jacksonian fits, HHE syndromes (hemiconvulsions hemiplegia, epilepsy [3]) or absences were noted.

— Focal spikes or sharp waves in other regions without disturbed background activity

One of the characteristic features of the Rolandic spikes is the normal background activity which is regularly found. It was considered of interest to compare this group with those patients in which the EEG recordings did not show the strictly central localization of the discharges but a normal background activity A typical example is found in Fig. 2

We found only 6 such patients, 3 boys and 3 girls, age range 2–12 years when the first EEG was made. The clinical findings in these patients are summarized in Table 2 which is arranged in the same manner as Table 1 In the table the localization of the discharges is also indicated.

TABLE 1 *The Rolandic spike group*

l left; = right; f = female; m = male

Sex	f	f	f	f	f	f	f	f	m	m	m	m	m	m	m	m	m	m	m	m	m	m	m	m	m	m
Year of birth	1957	56	53	–56	–54	–51	–53	–48	–61	–57	–55	–59	–59	–53	–55	–53	–56	–53	–51	–49	–49	–49	–49	–50	–48	–47
Age at exam.	4	4	6	6	7	7	10	12	1	2	3	3	4	6	6	6	6	6	8	10	11	11	11	12	13	15
Dominant side of spikes	l			l	l	l		l	l	l	l		l	l			l			l	l		l	l	l	l
Type of seizure																										
N seizures																										
Generalized, symmetrical																										
Asymmetrical																										
Psychomotor																										
Clinical findings																										
CP hemiplegia																										
CP other types																										
Mental retardation																										
Malformation																										
Tumour CNS																										
Paroxysmal symptoms (i.e. headache, vertigo)																										

TABLE —. *Focal spikes or sharp waves with normal background activity*

f-t = fronto-temporal; t = temporal; t-o = temporo-occipital; o = occipital

Sex	f	f	f	m	m	m
Year of birth	1959	–62	–50	–57	–5	–47
Age at exam.	3	10	13	2	11	11
Focal region	f-t	o	t	t-o	t	f t
Type of seizure						
Generalized, symmetrical						
Psychomotor						
Minor motor						
Clinical findings						
CP hemiplegia						
Mental retardation						
Degen. disease						

It was found that all these patients had attacks. One of them had a mental retardation of doubtful origin (possibly toxoplasmosis cong.) and one a CP syndrome In four of the patients the seizures were the only finding.

3. *Focal spikes or sharp waves displayed against a focal abnormal background activity*

We wanted for comparison a group where it could reasonably be believed that the EEG pattern was consistent with a more circumscribed cortical lesion. Therefore we did not include those cases where the discharges as well as the abnormal background activity spread over greater parts of one hemisphere, well aware of the fact that some of these patients might by other examiners have been regarded as having focal discharges. A typical example is found in Fig. 3

Sixteen patients belong to this group 10 boys and 6 girls, age range 1–16 years. The clinical findings are summarized in Table 3

Only one of them, a girl with CP (hemiplegia), had never had any seizure. In 7 patients the seizures were the only clinical finding It is notable that all the 8 pa

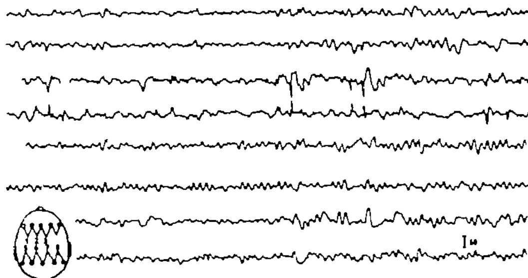


Fig. 3. Bipolar recording. 21 electrodes, patient awake. Focal spikes from right fronto-temporal region with abnormal background activity Patient no. 14, Table 3.

TABLE 3. *Focal spikes or sharp waves with abnormal background activity*

t temporal; – central; f – frontal; f t – fronto-temporal, p – parietal; – occipital

Sex	f	f	f	f	f	f	m	m	m	m	m	m	m	m	m	m
Year of birth	1963	53	–61	–61	–48	–49	–42	–57	53	–33	–52	–51	–46	–49	–47	–46
Age at exam.	7	7	7	9	10	14	1/11	3	7	9	10	12	13	13	13	16
Focal region	t			f-t	f	f-t	p	t	f	p	o	t	t	f-t		t
Type of seizures																
No seizures																
Generalized, symmetrical																
Asymmetrical																
Psychomotor																
Minor motor																
Clinical findings																
CP hemiplegia																
CP other types																
Mental retardation																

tients with a story of disease in the central nervous system beyond the seizures were CP cases. No case of tumour paroxysmal headache or vertigo was found in the group.

Comments

The reason for the division of the material into the three described groups was to see if the Rolandic spikes occurred in patients with a different clinical picture than that seen in children showing other types of focal discharges in their EEG s. W had the general impression that the Rolandic spikes were more benign This impression turned out to be rather dubious. 14 of the 26 patients in the Rolandic group had had seizures of some kind. Among th 12 without seizures were a few with paroxysmal headache or vertigo without signs of structural damage of the central nervous system [cf. 4]. However we also found cases with CP one case of agenesy of the callosal body and even one with a tumour in the posterior fossa [cf. 6] In this context it should also be noted that focal discharges (in two cases presumably of this kind) were described by Skadtvedt & Lundervold [7] in three cases of CP who prior to the first EEG never had seizures but developed such later

A small number of patients were found, whose EEG s contained discharges with the same characteristics as the Rolandic spikes, but with another localization, mainly occipital or temporal. Almost the same variety of clinical diagnosis and seizure types was found as in the other groups, but it should be emphasized that the number of patients is small. It is of some interest to note that this EEG pattern evidently is not restricted to the Rolandic region only

When the Rolandic pike group was compared with those patients who had a disturbed background activity in the region where the spikes and sharp w ves were found, suggesting a restricted cor

tical lesion, it turned out that in the latter group 15 out of the 16 patients had seizures. This difference between the two groups is probably significant. It can however not be excluded that the difference in age distribution is of some importance. On the other hand CP cases, with or without hemiplegia, were frequent in both groups. In the whole series of 49 cases we found 17 with CP (around 35 %). We could not find any certain correlation between the different EEG patterns and the presence or absence of *seizures or hemiplegia.*

Only one tumour case was found, a boy with Rolandic spikes (in two different registrations) and a pons tumour. W recently observed one patient, not included in the present investigation, who had spikes in the Rolandic region (confirmed during corticography) against a disturbed background activity. On operation a hemangiomatous malformation in the Sylvian fissure was found. The malformation was situated at some distance from the spike focus, which could not be refound at corticography immediately after resection or in repeated EEG s the weeks following operation. The psychomotor attacks disappeared.

With regard to seizure types, generalized or partial motor attacks dominated in all groups. However five cases of psychomotor epilepsy with temporal focus were found in the third group of patients. Four of these patients were 12 years old or more.

Serial examinations were carried out in many but not all patients. Because the material is not homogeneous in this respect we cannot draw conclusions with regard to the prognosis.

The Rolandic spikes (with their typical mode of occurrence) are in our experience exclusively found in children. We have never seen them in adults. The fact that the background activity in the region is practically always normal and that the patients with seizures and this EEG pattern very often have generalized seizures may justify the assumption that they are projected from subcortical structures. This is to some extent supported by the two tumour cases, in which the expansive lesions were localized to areas outside the central region (pons and Sylvian fissure). A study of the reactivity *of these potentials upon peripheral stimuli* or motor and mental activities of different kinds may be of value in further evaluating their significance from the pathophysiological point of view.

We conclude from the material presented that the Rolandic spikes do not necessarily have any definite correlation with cerebral lesions and their localization. This EEG pattern can be found both in patients with and such without epileptic attacks. However it is so often found in patients with cerebral damage and epilepsy that it should not be termed "functional" or "without significance". As to the question whether neuroradiological studies should be performed or not our conclusion is that the clinical picture and the neurological findings in each patient must be the decisive factor.

Summary

A five year material of EEG registrations in children, consisting of *404 recordings from 1440 patients, was surveyed with reference to the occurrence of Rolandic spikes and their clinical correlation.

The authors found 20 typical cases of Rolandic spikes as compared with 22 cases of strictly focal abnormalities of epileptogenic types with other localisation.

The material was analyzed with regard to seizure types and other clinical findings. More than 50 % of the patients with Rolandic spikes had seizures. Furthermore, signs of structural damage of the central nervous system were so frequently found that a careful neurological examination followed by a prolonged observation time seems to be warranted in these cases. The EEG pattern only is however no indication for neuroradiological examinations.

References

1 BANCAUD, J COLOMB, D and DELL, M. B.: Les pointes rolandiques un symptôme propre à l'enfant. *Rev Neurol (Paris)*, *99* 206, 1958.
2. GASTAUT H., POIRIER, F PAYAN H., SALAMON G TOGA, M. and VIGOUROUX, M.: H.H.E. Syndrome. Hemiconvulsions, Hemiplegia, and Epilepsy *Epilepsia*, *1* 418, 1960.
3. GASTAUT, Y Un élément déroutant de la séméiologie électroencéphalographique: les pointes prérolandiques sans signification focale. *Rev Neurol (Paris)*, *87* 488, 1952.
4. LOISEAU, P GRIMMAUD P COHADON, S., VINCENT D and FAURE, J Les paroxysmes rolandiques en dehors de l'épilepsie. *Rev. Neurol (Paris)*, *111* 274, 1964.
5. LOISEAU P GUERIN, A. and COHADON S.: Infantile sleep epilepsy The problem of Rolandic spikes. *Electroenceph Clin Neurophysiol*, *14*: 710, 1962.
6. NAYRAC, P and BEAUSSART M.: Les pointes-ondes prérolandiques: expression E.E.G très particulière. Étude électroclinique de 21 cas. *Rev Neurol (Paris)*, *99* 201 1958.
7 SKATVEDT, M. and LUNDERVOLD A.: The significance of electro-encephalographic spike foci in patients with cerebral palsy without epileptic seizures. *Acta Paediat Scand*, *45*. 440, 1956.
8. SMITH, J B. M. and KELLAWAY P Central (Rolandic) foci in children: an analysis of 200 cases. *Electroenceph Clin Neurophysiol*, *17* 460, 1964.

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Urinary Oestrogen Excretion in Newborn Infants with Congenital Dysplasia of the Hip Joint

by DAGFINN AARSKOG KARL FR STØA and THOR THORSEN

An increasing body of evidence indicates that biologically active oestrogens are rapidly converted into inactive, or less active compounds by the newborn infant. However the pathways of oestrogen metabolism in the newborn differ from those in the adult in important qualitative, as well as quantitative interrelationships [5 12] It has been demonstrated that oestriol is excreted in vast quantities during the first few days of life, whereas the biologically active oestrone and oestradiol-1 β are present in hardly detectable amounts, even following the intramuscular injection of oestradiol in doses amounting to 500 μg [7]. This special hormonal milieu which is characterized by the virtual absence of the biologically highly active oestrone and oestradiol 17β is also maintained by the human foetus [6].

From a teleological point of view it has been suggested that this phenomenon may represent a protective measure against possible deleterious effects of the biologically active oestrogens. It was therefore of considerable theoretical interest when Andrén & Borglin in 1961 [ ] suggested that congenital dysplasia of the hip was due to an inborn error of oestrogen metabolism characterized by an impairment in the conversion of oestrone and oestradiol 17β into biologically inactive oestrogens. Owing to this metabolic error these infants were supposedly exposed to a greater relaxing effect of biologically active oestrogens on the pelvic tissues resulting in a relaxation of the hip joint capsules.

Andrén & Borglin based their hypothesis on the finding of an appreciabely higher excretion of oestrone and oestradiol 17β in the urine of newborn infants with congenital dislocation of the hip than in the normal newborn. They also reported that administration of oestradiol benzoate increased the exeretion of oestrone and oestradiol 17β in newborn infants with congenital dysplasia of the hip whereas such an increase could not be demonstrated in normal controls [3]

To our knowledge the findings reported by Andrén & Borglin have so far not been confirmed by other investigators. Andrén & Borglin used the relatively insensitive method of Brown *et al.* [4] in their oestrogen determinations. In recent years more specific and sensitive methods of oestrogen determination have

been developed. Employing such methods we have repeated the investigation of Andrén & Borglin regarding the urinary excretion of oestradiol-17β in newborn infants with congenital dysplasia of the hip joint. In addition, we have also studied the excretion of the α ketolic oestrogens.

Material and Methods

The material consisted of seven newborn infants with bilateral positive Ortolani' sign. There were six girls and one boy All infants were delivered by the agmal route. In three cases there was a family history of dislocation of the hip joints (cases J J., F S. and D B.) The control group consisted of four newborn infants selected on the basis of a history of normal vaginal delivery and normal findings on general physical examination. The infant D.B in the control group and the infant D.B with a positive Ortolani sign were monozygotic twins.

The urine specimens were collected during the first three days of lif by means of ad hesi colostomy bags of plastic. The method used for oestrogen analysis has been de scribed in detail by Stoa & Thorsen [11] and by Thorsen *et al.* [12]. Briefly t entails hydrochloric acid hydrolysis, ether extraction, sol ent partition m benzene/light petroleum/0.25 N sodium hydroxide, fol lowed by alumina column chromatography The oestradiol" fraction was separated into 16-keto-oestradiol 17β and oestradiol 17β by paper chromatography in the system monochlorobenzene/formamide. After elution, the oestrogens were measured fluorometrically according to the method of Itt rich [9] as described by Stoa & Thorsen [11].

The use of acid hydrolysis and treatment with alkali for purification will result in an almost complete transformation of any 16 α-hydroxy-oestrone to 16-keto-oestradiol 17β. [10]. The 16-keto-oestradiol-17β measured may therefore be considered t represent the sum of the ring D ketolic oestrogens present in the urine

Results

The results of the study are listed in Table 1 All the infants studied excreted only traces of oestradiol-17β and there was no difference between the two groups of newborn infants in the amounts ex creted. The infants showed greater individual variation in their excretion of ring D α ketolic oestrogens, but the amounts excreted were of the same order of magnitude in both groups of infants

Discussion

In quantitative studies of urinary ex cretion of steroids in newborn infants, there will always be uncertainty as to the completeness of the urine collection. However m our hands the relatively simple method of urine collection employed in the present study has been proven to be very efficient [1]

That newborn infants excrete only traces of oestradiol-17β is in accordance with earlier reports on the urinary x cretion of oestrogens in normal newborn infants. Andrén & Borglin s finding of an increased excretion of oestradiol-1 β in newborn infants with congenital dysplasia of the hip has not been confirmed in the present study Unfortunately And rén & Borglin report only the mean values and ranges found in their study [ ] However some individual values were reported in a subsequent study m which the urinary excretion of oestrone and oestradiol-17β was examined before and after the administration of oestradiol benzoate to the infants [3]. In 3 out of 11 cases, detectable amounts of oestradiol 17β were found, before the injection of oestradiol benzoate but in all instances

TABLE 1 *Urinary excretion of oestradiol-17β and ring D α ketolic oestrogens in the normal newborn and in newborn infants with congenital dysplasia of the hip joint.*

Group	Subject	Oestradiol 17β μg/24 hrs.			Ring D α-ketolic oestrogens measured as 16-keto-oestradiol 17β μg/24 hrs.		
		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Negative Ortolani sign	E. A.	Not detectable	0.57	Not detectable	6.40	1.40	1.08
	O. B.	Not detectable	0.21		1.00	0.52	
	E. T.		Not detectable	Not detectable		1.21	0.60
	D. B.		Trace			1.30	
Positive Ortolani sign	D. B.		Not detectable			0.37	
	F. R.		Not detectable			1.50	
	K. G.		Not detectable			4.80	
	J. J.			0.20			0.63
	B. K.			Not detectable			0.20
	S. L.			Trace			0.51
	J. P.			Not detectable			0.27

the amount excreted was at the lower limit of the sensitivity of the method used (0.5–1.0 μg/24 hours). It thus appears that the increased excretion of oestradiol 17β was not a constant finding in the infants studied by Andrén & Borglin.

The present investigation further substantiates our previous finding that the newborn infants excrete significant amounts of ring *D* α ketolic oestrogens [11]. This finding has recently been confirmed by Hagen *et al.* [8] who also reported the presence of other, as yet unidentified, hydroxylation products of oestradiol 17β in the urine during early infancy. It is possible that an extensive usage of hydroxylation reactions may represent a protective mechanism against biologically active oestrogens during intrauterine life, and that this special pattern of oestrogen metabolism persists for some time after birth.

In our opinion the concept of a hormonal influence on the development of congenital dysplasia of the hip joint remains an attractive hypothesis. However, so far conclusive experimental support for such a hypothesis has not been brought forward.

Summary

The urinary excretion of oestradiol 17β and total ring *D* α ketolic oestrogens has been studied during the first three days of life in seven infants with congenital dysplasia of the hip joint. Four newborn infants with normal findings on

general physical examination served as controls. There was no detectable difference between the two groups with respect to the amount of oestrogens excreted.

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References

1. Aarskog, D., Støa, K. F. and Thorsen, T. Cortisol metabolism in the newborn. Urinary excretion of free and conjugated compounds following intravenous administration of cortisol-4-^{14}C to full term and premature infants. *Acta endocr (Kbh.)*, *44*: 256, 1964.
2. Andrén, L. and Borglin, N. E.: Disturbed urinary excretion pattern of oestrogens in newborns with congenital dislocation of the hip. I. The excretion of oestrogens during the first few days of life. *Acta endocr (Kbh.)*, *37* 423, 1961
3. — Disturbed urinary excretion pattern of oestrogens in newborns with congenital dislocation of the hip. II. The excretion of exogenous oestradiol-17β *Acta endocr (Kbh.)*, *37* 427 1961.
4. Brown J B., Bulbrook, R. D. and Greenwood, F C.: An additional purification step for method for estimating oestriol, oestrone and oestradiol-17β in human urine. *J Endocr* *16* 49, 1957
5. Diczfalusy E. and Lauritzen C *Oestrogene beim Menschen*. Springer Verlag, Berlin, 1961.
6. Diczfalusy E. and Magnusson A. M.: Tissue concentration of oestrone oestradiol-17β and oestriol in the human foetus. *Acta endocr (Kbh.)*, *28* 169 1958.
7. Diczfalusy E., Tillinger, K. G and Westman A.: Studies on oestrogen metabolism in newborn boys. I. Excretion of oestrone, oestradiol 17β and oestriol during the first few days of life. *Acta endocr (Kbh.)*, *26*, 303, 1957
8. Hagen, A. A., Barr, M. and Diczfalusy E.: Metabolism of 17-oestradiol-4-^{14}C in early infancy *Acta endocr (Kbh.)*, *49* 207 1965.
9. Ittrich, G Eine Methode zur chemischen Bestimmung von östrogenen Hormonen in Blut, Milch und Colostrum. *Hoppe-Seylers Z physiol Chem*, *320* 103, 1960.
10. Marrian G F The biochemistry of the oestrogenic hormones. *IVth International Congress of Biochemistry* Vienna. Symposium No. IV 1958.
11. Støa, K. F and Thorsen T Fluorimetric determination of oestrogenic steroids using Ittrich's extraction method. *Acta endocr (Kbh.)*, *41* 481 1962.
12. Thorsen T Støa, K. F and Aarskog, D.: Identification of ring D ketolic oestrogens in the urine of newborn infants. *Acta endocr (Kbh.)*, *45* 416, 1964.

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β Glucuronidase Activity in the Serum of Infants

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Glucuronide metabolism has received much attention because of its relationship to neonatal jaundice [1 2 3 4]. The development of hepatic conjugation mechanisms has been extensively studied [1 2] but the development of β-glucuronidase activity has been followed in the liver of mice only [5] It has been reported [5] that β-glucuronidase activity is significantly higher in newborn and infant mammals than in adults. In previous work it has been shown that in the rat [6] mouse and rabbit [7] ileum there is a rise in β-glucuronidase activity after birth (using phenolphthaleinglucuronide as the substrate) and a fall after weaning Later it was found [8] that liver β-glucuronidase activity (phenolphthaleinglucuronide served as the substrate) in newborn rats was significantly lower than in adult animals, in rabbits values increased up to day 3 and in mice only small developmental changes were found. This was in contrast to the finding of Karunairatnam *et al* [5] who used phenylglucuronide as the substrate, and it was concluded [8] that the opinion voiced repeatedly in the literature that infant animals tend to have a higher β-glucuronidase activity in the liver applies to mice only and only if phenylglucuronide is used as the substrate These findings and the preliminary report of Dutton *et al.* [9] indicated that glucuronide synthesis could be high in some species during the neonatal period and not low as has been judged earlier [cf. 1 2 3 4 9] and led us to study the postnatal changes of β-glucuronidase activity in infants.

This report describes results of estimation of β-glucuronidase activity in serum.

Methods

Activity was determined in a modification of the method described by Talalay *et al.* [10]. Phenolphthaleinglucuronide (0.001 *M*) served as the substrate, and the pH was adjusted to 4.5 in 0 1 *M* acetate buffer—after first showing that this was the optimum pH. To ensure a linear relation between the amount of serum added and liberated phenolphthalein the serum was diluted with bidistilled water 1 15 (cf Pineda et al. [11] and Fazekas [12]). Incubation was carried out at 50°C for 48 hrs (the linearity of the reaction was tested).

The blood serum specimens were obtained from healthy children born from normal pregnancies and from adult persons in the morning hours. The experimental subjects were not fasted. Sex distribution was equal.

Results

The figure shows that the activity in newborns during the two first days is low

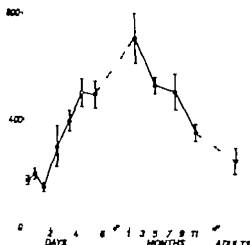


Fig. 1. *Ordinate.* β-glucuronidase activity expressed in μg phenolphthalein liberated per ml of serum per 24 hours at 50°C. *Abscissa.* days (age in days); months (age in months); adults (adult persons 20–40 years old). Each circle is the average of determinations of at least 10 persons. Vertical lines denote ± S.E. Since no sexual differences have been found, values for both sexes were combined. The following differences are statistically significant at least for $p < 0.01$ 6 hour-old, 16-hour-old and 36-hour-old against 2-day-old, 4-day-old, 5-day old, 6-day-old, 1 3-month-old 4–6-month-old and 7–9-month-old; adult against 1 3-month-old, 4–6-month-old, 7–9 month-old, 5-day-old, 4 day-old, 2-day-old, 36-hour-old and 6-hour-old.

there is a rise from the second to the 6th day of life. The highest values were found in the serum of 1–3-month-old children During the first year of life a steady decrease was observed. The values for children aged 10–1 months were on the same level as adult values and were near to values found in 2-day-old infants.

Discussion

Our results show that in the first days of life low β-glucuronidase activity is found in the serum. These results are in good correlation with our other results on experimental animals [6, 7 8], where during the first postnatal days low activity of β-glucuronidase in the ileum, jejunum and liver was found. It is of course clear that more studies—with other substrates and on more pertinent tissues for the metabolism of glucuronides — liver etc.— are needed for elucidation of the problem of the metabolism of glucuronides.

Summary

The β-glucuronidase activity (substrate phenolphthalein glucuronide) in the serum of infants increases during the first 6 days of life. After the maximum at 2 months a steady decrease is observed. The values of 1 year-old children are as high as values of 2-day-old infants and do not significantly differ from values observed in adult persons.

References

1. Symposium on Kernicterus. *IX International Congress of Paediatrics, 1959* University of Toronto Press, Toronto, 1961
2. Symposium on foetal and infant liver function and structure. *Ann N Y Acad Sc, 111* 1 1963.
3. Marsh, C. A. and Levvy G A.: The metabolism of D glucuronic acid; in D P Cuthbertson (ed): *Progress in Nutrition and allied Sciences.* Oliver and Boyd, Edinburgh and London, 1963, p 307
4. Conchie, J and Levvy G A.: Mammalian glycosidases and their inhibition by aldonolactones. Ibid. p. 313.
5. Karunairatnam, M. C., Kerr, L. M. H. and Levvy G A.: The glucuronide synthesizing system in the mouse and its relationship to β-glucuronidase. *Biochem J 45* 496, 1949
6 Heringová, A., Jirsová and Koldovský O Postnatal development of β-glucuronidase in the jejunum and ileum of infant rats. *Canad J Biochem, 43* 173, 1965.
7 Jirsová, V Koldovský O Heringová A, A

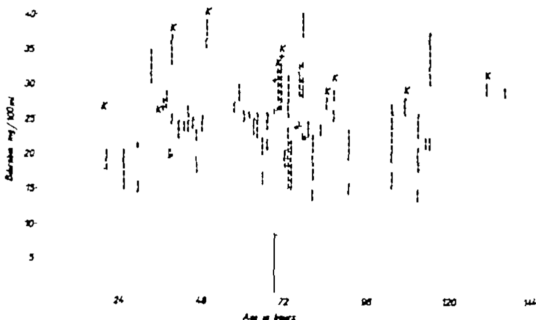


Fig. 1 Conjugated and unconjugated bilirubin values in 50 cases of haemolytic disease of the newborn. —— unconjugated bilirubin; - - - conjugated bilirubin; + death; *K* kernicterus.

ted bilirubin were found. The haemolytic disease was due to Rh incompatibility in 61 cases and to ABO incompatibility in 6 cases. The amount of conjugated bilirubin ranged from 2.1 to 21.6 mg/100 ml. Bilirubin levels in 50 infants in whom the concentration of total bilirubin exceeded 20 mg/100 ml are shown in Fig 1. A group of 17 cases with a total bilirubin level under 20 mg/ml was omitted. No cases of kernicterus occurred in this group.

There were 10 cases of kernicterus (3 deaths and 7 survivals) and 4 deaths from other causes (1 prematurity bronchopneumonia, 2 severe anaemia, haemorrhagic diathesis, 3 severe anaemia, umbilical sepsis, 4 heart failure during the exchange transfusion). The most important finding was that the kernicterus did not occur in infants in whom the maximum concentration of unconjugated bilirubin was less than 20 mg/100 ml.

Table 1 indicates that the incidence of kernicterus was significantly dependent upon the concentration of unconjugated bilirubin, while the level of conjugated bilirubin apparently does not play any *direct* role. The average level of total bilirubin was a little higher in the group with high value of unconjugated bilirubin, the difference was significant.

There were marked clinical differences between both groups. Most infants with low values of unconjugated bilirubin (group A) showed the typical inspissated bile syndrome with severe anaemia and highly increased conjugated bilirubin level apparently without risk of kernic-

TABLE 1 *Evaluation of relationship between fractions of bilirubin and clinical outlook.*

V	Unconjug. bilirubin level	Conjug. bilirubin mean level[a] (mg)	Total bilirubin mean level (mg)	Kernicterus[c] %	Deaths from other causes	Severe anaemia Hb < 10 g/100 ml %
Group A 26	< 20 mg/100 ml	11.29 ± 5.01	25.80 ± 4.65	0–0	2–7 7	64.0
Group B 24	> 20 mg/100 ml	4.00 ± 2.25	28.50 ± 3.97	10–41 7	2–8.3	12.5

SED = 1.11 mg $p < 0.01$. [b] SED = 1.25 mg, $p < 0.05$. $\chi^2 = 12.00$, $p < 0.01$.

terus. Infants of group B were not anaemic and the slightly increased level of con jugated bilirubin was obviously a com plication of the steep rise of unconjugated bilirubin which implied the risk of ker nicterus.

A comparison between normal and damaged infants of group B (Table 2) shows higher levels of both conjugated and un conjugated bilirubin in kernicterus cases but only the difference between mean values of total bilirubin tends to reach the 5 % significance level (D/SED = 1.94) Subgroups are too small to allow any conclusions on the enhancing role of conju gated bilirubin in infants in whom the dangerous level of unconjugated bilirubin is present.

Discussion

Icteric newborn infants with a high proportion of conjugated bilirubin rarely present difficulties when the need for the *first* exchange transfusion is assessed. The early presence of conjugated bilirubin is almost invariably associated with severe anaemia or a rapid rise of bilirubin, and these situations form the obvious indica tion for exchange transfusion. On the other hand, the secondary accumulation of conjugated bilirubin after the exchange transfusion, which may raise the level of total bilirubin considerably and keep it high for several days, may cause doubt as to whether the infant is threaten ed by kernicterus or not. It is possible that in some resistant cases of haemo-

TABLE _ *Comparison of bilirubin fractions between normal and kernicterus cases*

	V	Unconjug. bilirubin mean level[a] (mg)	Conjug. bilirubin mean level (mg)	Total bilirubin mean level[c] (mg)
Kernicterus	10	25.35 ± 3.83	5.09 ± 2.97	30.44 ± 4.83
Normal infants	14	23.73 ± 2.47	3.39 ± 1 13	27 11 ± 2.71

SED 1 34 mg, D/SED 1 19 SED = 1.04 mg, D/SED = 1.64. SED = 1 71 mg, D/SED = 1.94.

lytic disease requiring repeated exchange transfusions, as reported in the literature only the level of total bilirubin was used as the base for clinical assessment.

Our observations have shown that the risk of kernicterus in infants with increased concentration of conjugated bilirubin is minimal, unless the level of unconjugated bilirubin reaches the height which has been empirically estimated as the limit of safety. Our materials do not answer the question of whether the presence of a larger amount of conjugated bilirubin could enhance the genesis of kernicterus after the level of unconjugated bilirubin exceeded this limit.

Theoretically this possibility cannot be excluded. Electrophoretic studies [2] have shown that the conjugated bilirubin is as closely bound to serum albumin in extracellular fluid as is the unconjugated fraction, however the mode of its binding is less clearly understood and nothing is known about the possibility of its competition with unconjugated bilirubin for binding sites on albumin, as has been proved for other anions. The restriction of binding capacity of extracellular fluid for unconjugated bilirubin by such a competitive mechanism could increase the danger of development of kernicterus.

Our present practice is to ignore the presence of conjugated bilirubin unless its level exceeds 2 mg/100 ml assuming that a larger "zone of safety" is gained when the total bilirubin value is used as the principal criterion of necessity of exchange transfusion. Larger amounts of conjugated bilirubin are subtracted from total bilirubin values. However we do not allow the level of unconjugated bilirubin to exceed the limit of 18–20 mg/100 ml.

Summary

In a series of 67 erythroblastotic newborn infants with increased level of conjugated bilirubin, there were 10 cases of kernicterus, all in infants in whom the concentration of unconjugated bilirubin exceeded the limit of 20 mg/100 ml. The presence of an increased level of conjugated bilirubin appears to be of no direct importance for the genesis of kernicterus, but its enhancing influence in infants with dangerous concentration of unconjugated bilirubin cannot be excluded. Clinical implications are indicated.

References

1. JIRSOVÁ, V., JIRKA, M. and JANOVSKÝ, M.: Importance of quantitative determination of direct and indirect bilirubin in haemolytic disease of the newborn. *Acta Paediat. Scand.*, *47*: 179, 1958.
2. KLATSKIN, G. and BUNGARDS, L.: Bilirubin-protein linkages in serum and their relationship to van den Bergh reaction. *J. Clin. Invest.* 35: 537, 1956.
3. ZUELZER, W. W. and BROWN, A. K.: Neonatal jaundice. *Am. J. Dis. Child.* *101*: 87, 1961.

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Vaccination with Attenuated Type 1 Poliovirus, the Chat Strain

I A Study of 20 Families

by MARGARETA BÖTTIGER, SVEN GARD and RUTGER LAGERCRANTZ

In 1957 i.e. in the same year as large-scale parenteral vaccinations with inactivated poliovirus vaccine were introduced in Sweden, the first trial with attenuated live oral vaccine was initiated on a selected group comprising 20 families.

The aim of this study was to obtain information on the immunologic response, duration of immunity and excretion and spread of vaccine virus after oral vaccination in individuals pre-vaccinated by the parenteral route. Further this trial provided material for the evaluation of the genetic stability of the vaccine virus after passages in the human intestines [5]. Preliminary data from this experiment were reported at the First and Second International Conference on Live Poliovirus Vaccines [9 10].

Material and Methods

Vaccines. 1 Inactivated trivalent poliovirus vaccine produced in 1957 by the National Bacteriological Laboratory Stockholm [4].

2. Live Type 1 poliovirus vaccine strain Chat, batch 10A11 [12] prepared in monkey kidney tissue at the Wistar Institute Pennsylvania.

Serological tests. Blood samples were collected from finger puncture. 0.3 ml of the blood specimens was immediately diluted in 1.2 ml of a physiologically balanced heparin solution. Antibody titrations were performed by Gard immuno-inactivation technique as described previously [4, 8]. Each serum dilution was inoculated into 4 tubes.

Virus isolations. Extracts of stool specimens were prepared by mixing approximately 1 gram of feces with 10 ml of distilled water The mixture was shaken vigorously and centrifuged for 1 hour at 3500 r.p.m. Antibiotics were added to the supernatant and 0.2 ml was inoculated into each of 4 roller tubes containing a monolayer of primary monkey kidney cells. After a 2-hour incubation at 37°C the medium was changed. Parker 199 supplemented with 0.2 per cent bactotryptose and antibiotics, was used as maintenance medium. Tubes were rolled at 37°C and read for cytopathogenic changes after four seven and ten days of incubation. Blind passages were made of all cultures with equivocal changes.

Plan of the study Twenty-one families enrolled in the Norrtull Well Baby Clinic, Stockholm, participated in the study Each family had two or more children in the preschool age (below 7 years of age) The total was 47 children and 43 adults.

Vaccinations beginning in November 1957

were carried out and samples collected according to the following schedule:

The first blood sample was collected from all members of the household, after which everyone was given two subcutaneous injections of inactivated vaccine at one month's interval.

The antibody response was tested serologically. Persons still negative to Type 1 were revaccinated. When all members of the household were serologically positive to Type 1 6 log TCID$_{50}$ of live virus vaccine was fed to one child in 13 of the families and 4.5 log$_{10}$ TCID$_{50}$ to one child in four of the families. Among the latter group there was one who failed to excrete virus. This child was revaccinated with 6 log TCID$_{50}$ and then became infected. These orally vaccinated children will be referred to as "index" children. One family took part in the first phase of the study only. In two other families all oral vaccinations were postponed till the second phase of the study. This circumstance and the impossibility of contacting all family members on all occasions, explains irregularities in the number of test samples on various occasions.

Virus excretion in the stools was followed in all members of the household. Weekly specimens were collected as long as any member excreted virus. Blood samples were again collected 2 months and 8–10 months after the feeding of the index child. Immediately after the latter sampling the whole family including the index child, was challenged with live attenuated virus. Excretion was again studied in weekly stool specimens and blood samples were again collected after 2 months. Blood samples were collected about 3 years later from the majority of the families and 5 years later from 47 of the participants.

Results

Antibody status before and after parenteral vaccination. The immunity status of the family members prior to and after the parenteral vaccination is shown in Table 1.

In pre-immunization tests 30 of the 47 children and 4 of the 43 adults were found to be triple-negative. All children except two lacked demonstrable antibodies at a 1:10 dilution to poliovirus Type 1. The two children with titers of 1:10 were infants with probable maternal antibodies. Ten of the children (none of the index children) had received two doses of inactivated vaccine already in the spring of 1957. The only trace of this was seropositivity to Type 2.

After two injections with inactivated vaccine 11 individuals, mainly children, still lacked demonstrable antibodies to

TABLE 1. *Antibody titers prior to and after vaccination with inactivated poliovirus vaccine.*

Figures in the headings represent reciprocals of serum dilutions. The values in the table represent the number of individuals having the indicated serum antibody level.

	Prior to vaccination							After vaccination					
		Antibody titer							Antibody titer				
	Type of virus	<10	10	50	250	1250	>6250	Type of virus	<10	10	50	250	1250	>6250
Children	1	43	2					1	2	13	12	12	5	
	2	33	6	5	3			2		2	11	14	13	4
	3	43	2	1	1			3	6	19	8	9	2	
Adults	1	14	2	3	8	1	2	1		3	4	7	17	10
	2	13	4	3	7	11	3	2		3	7	8	15	9
	3	19	3	7	10	3	1	3	6	8	6	2	5	13

TABLE .. *Distribution of antibody titers to poliovirus Type I at various points of time.*

The different groups are explained in the text and in Fig. 1 Figures in the heading represent reciprocals of antibody dilutions and letters at the side the following times *A* prior to vaccination; *B*, after parenteral vaccination; *C* 2 months after the *feeding* of the index children; *D*, 10 months later prior to the general oral vaccination; *E*, 2 months after the oral vaccination; *F* 3½ years later

Group I

	<10	10	50	250	1250	6250	31250
A	25	1					
B	2	8	6	6	4		
C			4	7	11	1	
D	1	1	7	11	5		
E				1	16	8	
F			6	14	3	1	

Group II

	<10	10	50	250	1250	6250	31250
A	19						
B	1	5	5	6	2		
C	3	7	2	4			
D	4	6	7	1			
E			2	1	10	4	1
F		1	6	7	2	1	

Group III

	<10	10	50	250	1250	6250	31250
A	13						
B		3	3	6	1		
C	2	1	5	5			
D	3	1	5	4			
E	1		6	4	1		
F	1	5	2	4			

Group IV

	<10	10	50	250	1250	6250	31250
A	1	3	3	6	9	1	1
B				2	11	9	
C			1	3	4	9	1
D			2	1	10	8	1
E				3	15	4	
F			1	5	9	5	

Probable maternal antibodies.

Type 1 These individuals were given a third injection. The post-vaccination status after two (or three) injections, is shown on the right of Table 1

Antibody response to oral vaccinations

For the evaluation of the serologic response to the live Type 1 vaccine the material was divided into four groups.

I. Children excreting virus after the first feeding, i.e. index children and infected siblings (n = 26)

II. Children not excreting virus in connection with the first feeding (n = 10) All the children in groups I and II could be regarded presumably as naturally non-immune to poliovirus Type 1 before vaccination.

III. Adults without demonstrable pre-immunity (n = 13)

IV Adults with demonstrable pre-immunity (n = 22)

The distribution of antibody titers to Type 1 at various times throughout the study is shown in Table and the geometric mean titers expressed as positive logarithms to the base 10 in Fig 1 Titers below 1 10 were recorded as zero. The first values at "A" represent the pre-vaccination immunity status. Groups I–III had no detectable antibodies in the dilution 1 10, and group IV (adult pre-immunes) had a mean level of *.5 $\log_{10}$ (1.320)

After the immunization with parenteral vaccine (B) all the four groups reacted with titer rises.

Gro p I i.e children excreting virus after the first feeding, showed a considerable increase in titer 2 months after their first contact with live virus (C) (1 73 to .07 $\log_{10}$) During the following 8-month period a mean titer decrease of about 0.5 $\log_{10}$ (D) was observed.

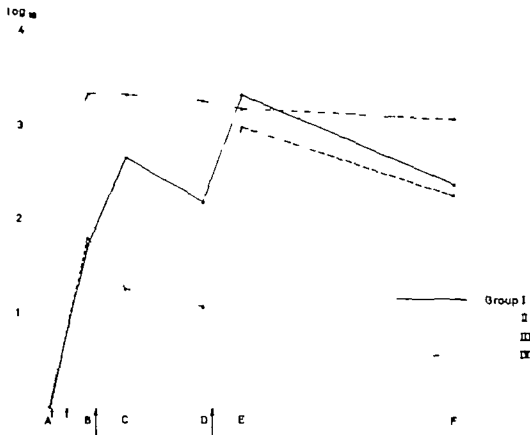


Fig 1 Geometric mean titers of serum samples collected at different times throughout the study On the ordinate the mean titers of groups I to IV are plotted. Group I index children and infected contacts, group II = children not excreting virus in connection with feeding of the index child (first feeding) group III = adults without demonstrable pre-immunity and group IV adults with demonstrable pre-immunity On the abscissa the various points of time at which the blood samples were collected are indicated. *A* pre-vaccination samples *B* after inoculation with inactivated vaccine *C* 2 months after feeding of the index child; *D* 7–8 months later; *E* 2 months after general oral vaccination, and *F* 3 years later Small arrows indicate inoculations with inactivated vaccine and big arrows feeding with live vaccine

Two months after the second challenge (E) with live virus the children in this group were again found to react with considerable titer rises, reaching the mean level of 3.3 $\log_{10}$. This value even exceeded the level of the naturally preimmune adults (group IV) During the following 3-year period (F) the values again decreased, approximately by 1.0 $\log_{10}$.

Group II In the children who did not excrete virus in connection with the first phase of the study the fall of titers after parenteral vaccination is clearly demonstrated in Fig 1 First there appeared a relatively rapid decline (B–C) followed by a slower (C–D) The average decrease of titers during the first two months was estimated to be 0.6 $\log_{10}$. During the following 8 months the fall was not quite 0.2 $\log_{10}$. On challenge with live virus they

responded very strongly and showed even higher titer rises than the index children had done after their first feeding (E). The follow up results, 3 years later actually differed very little from those of children challenged twice with live virus (group I) (F).

Group III The adults without demonstrable pre-immunity prior to vaccination reacted less actively to the challenge with live virus than did the children. The following decline of titers, however was relatively similar to that of the children. One pre-vaccination sero-negative person, although excreting virus for 5 weeks, did not react with any antibody rise. She was sero-negative to Type 1 in both her post-vaccination tests. To poliovirus Type 3 she had titers of 1.1-50 however

Group IV The naturally pre-immunes did not appear to respond at all to the challenge with live virus. The rate of decline of titers did not exceed 0.1 $\log_{10}$ per year

No fall of titers was observed between the three- and five-year post-vaccination samples of the 47 vaccinees, children and adults, investigated 5 years after vaccination (not included in the figure).

Relationship between pre and post feeding titers

The relationship between the individual titers prior to and two months after oral vaccination is shown in Fig. _ The individuals were divided into the following four groups:

1. Children presumably having their first experience of live virus, vaccinees and contact-infected (=44)
2. Children challenged with live virus a second time ($n = 36$)
3. Adults without demonstrable pre-vaccination immunity ($n = 15$)
4. Pre-vaccination immune adults ($n = 25$)

In the first group the majority of the children with pre vaccination titers of 1:250 (-4 $\log_{10}$) or lower responded with titer rises to about 1 1250 (3.1 $\log_{10}$). At pre-vaccination levels of 1 1250 an antibody decrease after * months was noted in four out of five children. The pattern was the same for children in group 2. The adults primarily without demonstrable immunity (group 3) responded irregularly to the virus challenge.

Finally in the group 4 adults almost all had pre-vaccination titers of 1 1250 (3 1 $\log_{10}$) or higher and no increase was noted.

Excretion of and resistance to oral vaccine

The excretion of the vaccine virus, especially in relation to the pre-immune status of the vaccinees, was evaluated from the following groups:

1. Children presumably without previous contact with live virus (only vaccinees) ($n = 38$)
2. Children with one contact with live virus ($n = 23$)
3. Adults presumably without contact with live Type 1 virus, i.e. primary titers lower than 1 10 ($n = 15$).
4. Naturally immune adults ($n = 26$)

The excretion patterns of the different groups are recorded in Table 3. Each group has been further divided according to the level of circulating antibodies immediately prior to the oral vaccination

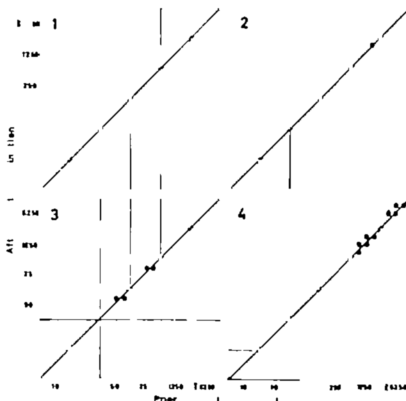


Fig. 2. Correlation between pre- and post-feeding titers in the four groups. Filled circles represent virus excretors and open circles nonexcretors.

The figures represent the number of individuals with the excretion periods indicated. The correlation coefficient (r) between the pre feeding antibody levels and the duration of excretion was estimated and included in the table. Significance levels (p) and the regression coefficient between the two factors (b) are also inserted.

Although the numbers of persons in the different groups were small some trends could be discerned.

All children with parenterally induced immunity (group 1) were found to excrete the vaccine virus. Individuals with lower antibody titers however tended to have a longer excretion period, although there were exceptions. Group 3 children i.e. those with one previous exposure to live virus showed considerably shorter excretion periods than group 1 children, even than those at the lower pre-feeding antibody levels.

Virus could not be isolated in 5 out of 15 individuals of the group 3 adults, of whom none had pre-vaccination antibody levels higher than 1:250. Although these individuals presumably had no prior contact with live virus, they may have possessed natural pre-immunity or resistance.

The naturally immune adults (group 4) were markedly resistant to the virus

TABLE 3. *Duration of excretion of vaccine virus in relation to pre feeding antibody titers*

Titers are given as reciprocals of neutralizing serum dilution. Figures in the table represent number of individuals at given excretion period. Statistical data are included. For the calculation of the regression the 5 logarithms of serum titers were used. Correlation coefficient between excretion periods and pre-feeding titers = and its significance level = p. The regression of duration of excretion/pre-feeding titer = b_1 and prefeeding titer/duration of excretion b_2.

Group	Pre-vaccination titer	0	1	2	3	4	5	6	7	8	9	10	11	12	13	and p	b_1 and b_2
1	<10				1					2		1		1	1		
	10			3	1	4	1		2	2				1		−0.24	b_1 = −0.77
	50		2	1	1	3			2	2				1			
	250		1				1		1					1		p = <0.20 >0.05	b_2 = −0.09
	1250		1	1								1					
	6250																
2	<10				1												
	10			1												−0.58	b_1 −0.40
	50	1	6														
	250	2	8	1												p = <0.01 >0.001	b_2 = −0.84
	1250	2	3														
	6250																
3	<10				1		1			1							
	10	1					1									0.24	b_1 = −1.11
	50	3			1	1								1			
	250	1		3												p <0.20 0.05	b_2 −0.19
	1250																
	6250																
4	<10																
	10																
	50		1														
	250	1															
	1250	12		1													
	>6250	10	1														
1 3																−0.27 p = <0.05 0.01	b_1 = −0.92 b_2 = −0.08

challenge. In 23 out of 26 individuals no virus was isolated, and of the 3 excretors none excreted virus for more than _ weeks.

Spread of virus

Of the 19 index children, 7 became spreaders. Eight out of 35 presumably susceptible contacts (as calculated by the outcome of the subsequent challenge of all family members with live virus) became infected. The infection was demonstrated as early as one week after the start of the experiment in 7 of 8 cases. There was no correlation between the concentration of virus in the stools or the duration of the excretion periods of the spreaders and the tendency to infect their contacts.

There appeared to be a relationship, however between age of the index child and the tendency to spread. All 7 spread-

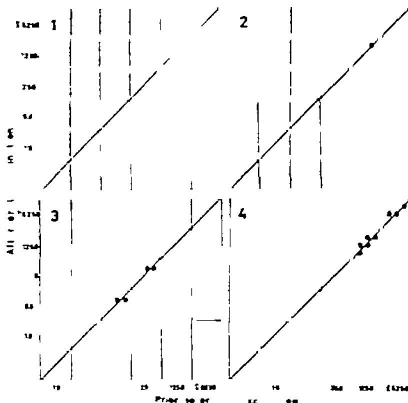


Fig. _ Correlation between pre- and post-feeding titers in the four groups. Filled circles represent virus excretors and open circles nonexcretors.

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Although the numbers of persons in the different groups were small, some trends could be discerned.

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Virus could not be isolated in 5 out of 15 individuals of the group 3 adults, of whom none had pre-vaccination antibody levels higher than 1:250. Although those individuals presumably had no prior contact with live virus, they may have possessed natural pre-immunity or resistance.

The naturally immune adults (group 4) were markedly resistant to the virus

post vaccination periods. Salk, Brown and coworkers [16, _] found similar results after immunization with inactivated vaccine only The post-vaccination levels in their studies remained almost unchanged between 1 and 6 years after the immuniza tion. When such follow up studies are evaluated, the eventuality of intercurrent wild virus infections must be taken into consideration. In the present study this possibility can almost be ruled out, as hardly any poliovirus isolations or suspected cases of poliovirus infections were reported from the area during the period.

The negligible post-vaccination antibody fall of the naturally immune adults is notable. This finding is in accordance with experience of immunization with bacterial antigens; after repeated boosters (which one may expect the naturally im mune adults to have experienced) the post-immunization antibody fall was successively reduced [1].

Excretion of vaccine virus

All vaccinees excreted virus after the first feeding in spite of pre-immunization with inactivated antigen. As many as 20 out of _3 vaccinees were found to be virus excretors after the second challenge with the live vaccine (performed 7-8 months after the first feeding) Although excretion periods were found to be con siderably shorter in the revaccinated, no absolute resistance to reinfection was thus noted. An almost total resistance to rein fection has been claimed by many investigators as the main advantage of the use of live vaccine [6 15].

As was pointed out in a preliminary presentation of this study in 1959 [9], there appeared to be an inverse correlation between pre-vaccination serum antibody level and duration of virus excretion. This was later confirmed by other investiga tors [11 22] Statistical analysis of the material, divided into groups as shown in Table 3 indicated that such a correlation existed but that the significance level was not higher than 80-85 per cent in the children orally vaccinated for the first time. Further analyses (variance analysis) showed, however that this group and the adult group presumably having its first contact with live virus did not diverge statistically and could be analysed together The increased material showed a correlation at the significance level higher than 95 per cent In the groups with pre vious contact with live virus a similar correlation was evident, the regression coefficient being of a quite different order however This latter fact limits the value of estimations of mixed populations (i.e with and without previous contact with live virus) with regard to correlation between virus excretion and antibody status.

As was mentioned above all children in this study with only parenterally induced immunity excreted virus. Dick and coworkers [7] later showed that parenterally induced antibody levels exceeding 1:220 inhibited or markedly reduced virus ex cretion. None of the children in this presentation had pre-feeding titers of this magnitude, however

Spread of virus

The age distribution of the spreaders indicated that children below * years of age were more pt to spread the infection than the older children. This period of change coincides with the age when chil dren generally learn toilet hygiene a fact

which might certainly contribute to restriction of the virus.

General remarks

This introductory experiment with live virus vaccine thus indicated a number of characteristics of its effect and behavior. The vaccine appeared to fulfil the requirements that could be demanded of an oral vaccine and that could be studied in small-scale trials. Thus its infectivity appeared sufficient to stimulate to antibody formation and its tendency to spread appeared limited (it was only transmitted from the youngest children).

As a result of these experiences the trials were continued and in the later studies [2, 3] special interest was directed to investigation of the infectivity and spread of the live vaccine in relation to the age of the vaccinee. Further estimations of the antibody response after combined vaccinations in different age groups were made.

Summary

Twenty families with children in the pre-school age were vaccinated both with inactivated and live (Type 1 Chat strain) poliovirus vaccine. All children with low pre-feeding antibody titers responded with titer rises after the oral vaccination. All of them had titers of or higher than 1:30 the majority 1:120 after the combined vaccination.

Higher post-feeding antibody levels (i.e. booster effects) were observed when live vaccine was administered 1 year compared to 2-4 months after initiation of immunization with inactivated vaccine.

The antibody response of the adults was less marked.

An approximately tenfold decrease of antibody titers was found during the 3-year period following vaccination in the children and pre vaccination non-immune adults. The titers of the pre-vaccination naturally immune adults remained more stable. Between samples collected 3 and 5 years after vaccination from both children and adults no further tendency to decrease was observed.

The duration of virus excretion was found to be inversely correlated to the pre vaccination antibody level and was markedly shorter in individuals with previous contact with live Type 1 virus. However as many as 20 out of 25 children still excreted virus after re-feedings performed 7 months after the first feeding.

Transmission of virus from vaccinees to their families occurred only from children less than 2 years of age.

References

1. Brown, C. G.: Duration of immunity. *Fifth Int. Poliomyelitis Conf.* Copenhagen 1960. J. B. Lippincott Co., p. 146.
2. Böttiger, M., Bottiger, E. and Zetterberg, B.: Vaccination with attenuated poliovirus Type 1, the Chat strain. III. Antibody response and spread of virus in schoolchildren. *Acta Paediat. Scand. 53*: 422, 1964.
3. Böttiger, M., Gard, S. and Zetterberg, B.: Vaccination with attenuated Type 1 poliovirus, the Chat strain. II. Transmission of virus in relation to age. *Acta Paediat. Scand. 53*: 416, 1964.
4. Böttiger, M., Arro, L., Lindberg, H. and Salmenstedt, C. R.: The immune response to vaccination with inactivated poliovirus vaccine in Sweden. *Acta Path. Microbiol. Scand.*, in press.
5. Böttiger, M.: Studies on characteristics of polioviruses. III. d, ret, A and e markers of

poliovirus isolated after vaccination with the Type 1 Chat strain. *Arch Virusforsch*, in press.

6. Chumakov M. P and coworkers On the course of mass immunisation of the population in the Soviet Union with the live virus vaccine from Albert Sabin's strains. *Fifth Int Poliomyelitis Conf* Copenhagen, 1960. J B. Lippincott Co., p. 228.
7 Dick, G. W A., McAlister, J Briggs, M. and Nelson, R.: Vaccination against poliomyelitis with the live virus vaccines. Effect of previous Salk vaccination on virus excretion. *Brit Med J II* 266, 1961.
8. Gard, S.: Immuno-inactivation of poliovirus. *Arch Virusforsch, 7* 449, 1957
9. Gard, S., Böttiger, M. and Lagercrantz, R.: Vaccination with attenuated poliovirus, Type 1 the Chat strain. *First Int Conf Live Poliovirus Vaccine, Pan Am San Bur* Sc. Publ. No. 44, 350, 1959.
10. Gard, S.: Field and laboratory experiences with the Chat strain type 1 poliovirus. *Second Int Conf Live Poliovirus Vaccines, Pan Am San Bur* Sc. Publ. No. *50* 187 1960.
11. Gendon, Yu Z. and Sanakoyeva, I. I.: Comparison of the resistance of the intestinal tract to poliomyelitis virus (Sabin's strains) in persons after naturally and experimentally acquired immunity *Acta Virol, 5* 265, 1961
12. Koprowski, H.: Discussion of Sabin, A., Properties of attenuated polioviruses and their behavior in human beings. *New York Acad Sc* (Spec. Publ.), *5* 113, 1957
13. Pettersson L. and von Magnus, H.: Antibody response to Type 1 oral polio vaccine. *Acta Path Microbiol Scand, 62*. 331 1964.
14. Plotkin, S. A.: The choice between killed and live virus poliomyelitis immunization. *Clin Pediat, 2* 220, 1963.
15. Sabin, A. B., Michaels, R. H., Spigland, I., Pelon W Rhim, J S. and Wehr, R. E.: Community wide use of oral poliovirus vaccine. *Am J Dis Child, 101* 546, 1961
16. Salk, J E.: Persistence of immunity following administration of formalin treated poliovirus vaccine. *Fifth Int Poliomyelitis Conf* Copenhagen 1960. J B. Lippincott Co., p. 157
17 — Vaccination against paralytic poliomyelitis. Performance and prospects. *Am J Publ Hlth, 45* 575, 1955.
18. Schär, M. and Goffe, A. P Controlled field trial with live attenuated poliomyelitis virus (Sabin type) in Switzerland. *Seventh Symp Europ Assoc Poliomyelitis and allied Dis*, Oxford 1961 p. 126.
19. Sohier, R. and Enders-Ruckle, G In-fluence of prevaccination immunity on the behavior of the polio vaccine type I (Sabin). *Eight Symp Europ Assoc Poliomyelitis and allied Dis*, Prague 1962, p. 511.
20. Wesslén T Lycke, E., Gard S. and Olin, G.: Inactivation of poliomyelitis virus by formaldehyde. *Arch Virusforsch, 7* 125, 1957
21. Wilson G S. and Miles, A. A.: *Topley and Wilson Principles of Bacteriology and Immunity* vol. II, p. 1347 1964.

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Vaccination with Attenuated Type 1 Poliovirus the Chat Strain

II Transmission of Virus in Relation to Age

by MARGARETA BÖTTIGER, SVEN GARD and BO ZETTERBERG

A first small-scale trial of immunization with inactivated poliovirus vaccine followed by live Type 1 "Chat" vaccine was performed in 1957 and 1958 in Sweden. This report concerns a similar study on a larger number of individuals carried out in 1959. The introductory family trial [1] suggested a higher tendency of children below 2 years of age to transmit the live vaccine virus than of older children. In the present investigations the spread of virus within the family in relation to the age of the vaccinees was studied. Continued testing of the serologic response was also carried out.

Material and Methods

Vaccines

The inactivated poliovirus vaccine was produced by the National Bacteriological Laboratory in Sweden [2, 13] and was inoculated parenterally. The live attenuated vaccine administered orally consisted of Type 1 Chat strain, batch 10A11 [5].

Antibody determinations

Titrations of neutralizing antibodies to poliovirus Type 1 were carried out as described previously [3]. Blood samples were collected by finger puncture and immediately diluted in a buffered heparin solution.

Virus isolation

Virus isolations were performed as described previously [1].

Plan of the study

The vaccinations were carried out at two places, one in the town of Eskilstuna and the other in the suburbs of Stockholm. All families were recruited from the Swedish middle-class community. The trials were carried out on similar schedules and will thus be recorded together. Some of the data from Eskilstuna, where Dr Lundström and Dr Kaiser conducted the local field trial and health control, have been reported earlier [ ].

The Eskilstuna trial included 64 families comprising 129 adults and 149 children. All families had 2 or more children, mainly in the pre-school age. The Stockholm vaccinations comprised 104 families, 10 adults (individuals older than 15 years) and 187 children. Seventy three of the 104 families had two or more children, the other 31 families had only one child.

A number of the participants, especially the children, had received two or three inoculations with inactivated vaccine prior to this study. Before the start of the experiment all unvaccinated subjects received 2 doses of inactivated vaccine while a booster injection was given to previously vaccinated subjects. The immunologic status was checked in blood samples collected one week after the last injection.

One child in each family the "index" child, was fed 6 log TCID$_{50}$ of Chat virus

In the 64 Eskilstuna families 32 of the index children were between 0–2 years old and 32 were about 4 years old.

All index children in the Stockholm area were older than 2 years; most were 4 years old.

In Eskilstuna stool specimens were collected in the first second and third week after vaccination, and thereafter monthly as long as any member excreted virus. In Stockholm the specimens were collected in the first, second and fourth week, followed by monthly specimens from families still excreting virus. Two months after the feeding of the index child blood samples were again collected from all members.

Throat swabs were collected from 43 vaccinees one week after the feeding.

The general health was followed by the Children Health Centers.

Results

Antibody response

The poliovirus Type 1 antibody status of all members of the families at the time of the feeding of the index child and 9 months later are recorded in Fig 1 The participants were arranged in three groups.

1 Index children and family members excreting virus.
2 Presumably non infected pre-school children (age <7 years)
3 Presumably non-infected schoolchildren and adults.

In the diagrams the antibody levels before the feeding of the index child are correlated to those observed months later The figures in the diagrams represent the number of individuals with the particular antibody pattern indicated.

The diagram of *group 1* indicates that all vaccinees had circulating antibodies to Type 1 after the combined vaccination. Irrespective of the pre feeding antibody status the majority ended up with titers of about 1 1250 (3 1 log$_{10}$) Thus vaccinees with lower pre-feeding titers showed relatively stronger antibody responses to the challenge with live virus The antibody status of individuals with pre feeding titers of 1 1250 and above appeared relatively unaffected by the virus challenge

The diagram of *group 2* illustrates the antibody levels of the presumably non infected pre-school children. The majority show unchanged or decreasing values at all levels during the 9 month interval

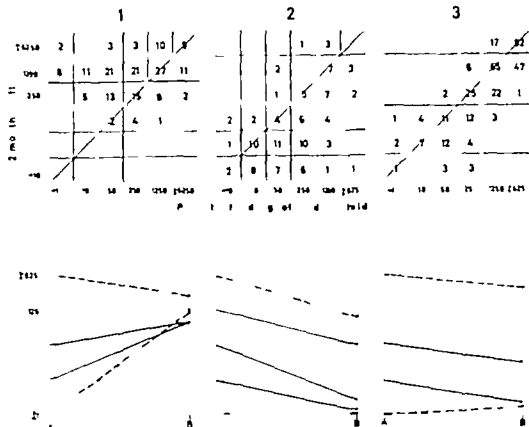
Group 3 the schoolchildren and adults, show the same pattern as group 2, the decline of titers being, however a little less marked.

The lower diagrams illustrate the geometric mean titers expressed in log$_{10}$ of the three groups on the two occasions

In group 1 the marked rise of titers of vaccinees with low pre feeding antibody levels and the apparently increasing insensitivity to the antigen challenge at pre-feeding titers of 1 1250 (3 1 log$_{10}$) and above is clearly illustrated The decline of titers in the other two groups, not fed or excreting virus, is also illustrated. It may be seen that the decline was probably a little more marked in young children (group 2)

Excretion of virus

In 8 of the 108 orally vaccinated children virus isolations failed. The pre-feeding titers of 7 of these 8 vaccinees amounted to 1 1250 (3 1 log$_{10}$) or higher the eighth had titer of 1 250 (4 log$_{10}$) Virus was isolated from feces of all vaccinees with antibody levels of 1 50 (1 7 log$_{10}$) or lower



F g 1 Correlation bet een serum titers of all family members prior to and subsequent to feeding li irus accine to the inde hild. The upper diagrams giv the data of the indi idual samples, the lower show the post feeding geometric mean alues of groups with different pre feeding antibody lev ls Diagram number 1 represents irus excretors, number 2 non-excreting pre school hildren and umber 3 non-excreting schoolchildren and adults. Dashed lines in the lower diagrams indicate that he pre feeding group alue might be either higher (&_50) or lower (10) than indicated in the figure A prior t feeding of inde hild, B months after feeding of index child.

TABLE 1 *Number of individuals excreting virus fter oral vaccination and the relation of excretion periods to pre feeding antibody levels*

Antibody titers are given as the reciprocals of the serum dilution

Pre-feeding ant body titer	Non excretors	Excretors: Weeks of xcretion 1		3–4	8	1	Total number
<10		1	1	1	4	6	20
10–50	1	4	1	28	14	6	75
1–0	6	14	11	26	5	1	63

The duration of virus xcretion in relation to the pre-feeding antibody tatus was roughly estimated, a survey of the excretion periods is given in Table 1 Individuals xcreting virus over long periods were found at all pre vaccination antibody levels. A relatively large number of non-excretors and of children with excretion periods of short duration were found however at the higher pre-feeding levels.

Virus was isolated from only one of the 43 throat swabs investigated.

TABLE 2. *Transmission of virus from vaccinees of different ages*

	0–< 2 years old		2 3 years old	
	Number of accinees	Spreaders	Number of vaccinees	Spreaders
Eskilstuna	22	11	30	
Suburbs of Stockholm			66	3
20 families in Stockholm	10	7	9	
T tal	42	18 (43 %)	10	5 (5)

Transmission of virus

The transmission of virus within the family was found to be related to the age of the vaccinees. The index children were divided into two groups. Group 1 com prised children less than years of age (born in 1958 or later) and group children about 4–5 years old (the majority of whom were born in 1955)

The results are given in Table 2. Data from the earlier study [1] are also presen ted. Only families with two or more children, of whom the index child ex creted virus, were included.

The tendency of vaccine virus to spread within the family was found to be almost 10 times higher in the group of vaccinees below years of age than in the older children.

Discussi n

Antibody response. The antibody response to the oral vaccination was similar to that found in the first study of pre-school children [1] The general equalization of titers, all children having titers of 1 50 (1.7 $\log_{10}$) and abov and the majority re aching 1 1–30 (3.1 log) was again found. It was also evident that children who responded weakly to the immunization with inactivated vaccine reacted actively to the challenge with live vaccine It would ha been interesting to compare these rea with a group of children receiving vaccine only after considera tion of the ible risk associated with the use of liv accine in previously non-immune ls, it was decided to use this vacc after previous immuniza tion with ated vaccine. Koprowski [6] repor mean antibody titer of 1 64 afte nization with Chat vac cine only corresponding group of pre-vaccina sero-negative children. Witt [14] and Voroshilova [13], in studies with the Sa Type 1 strain, also found a definite peak at 1 64. Similar results were obtained by de Pan [8]. Buser & Schar [4] reported somewhat higher ver age titers fter vaccination with the Chat strain only It must be remembered, how ever that the comparability of results of different laboratories is limited as long as not all titers are given with reference to a standard antibody unit. The possibility that sensitization after pre-immunization with inactivated vaccine helps to stimu late an increased ntibody response to challenge with live virus should be kept in

mind This question was discussed i previous paper [1]

From the serologic evidence there d e not ppear to have been any unn tic d wider pread of the vaccine viru within the families although this cannot be om pletely excluded in all instances

Transmission of virus

The transmission of virus fr m the children below 2 years of age w alm st ten times higher (43 per cent) than from children aged 4 years (o per cent) Since the age of 2 years coincides with the pe riod when children start toilet training it might be concluded that thi accom plishment contributes to a reduction of the spread of virus from fecal material Thu the tendency of increased viru transmission from children below ? years of ge as wa already indicated in the introductory tudy was further confir med. Such a trend can also be discerned in a similar study [4] carried out in Switzer land by Schär & Buser (personal com munication) In their material 13 of 3_ children below ? years of age were found to transmit virus, while only 1 out of 1 children, 3–9 years old was a source of infection In general the spread of virus appeared to be comparatively limited in thi tudy In a tudy of the vaccination of children of various ges in Poland [10] with the Chat strain carried out in a dis- trict with comparatively lower sanitary condition. 20 per cent of the adult contacts excreted virus. Sabin estimated that a transmission of his strains in an American field trial [11] occurred in up to 00 per cent of the contacts

The low frequency of isolation of virus from the pharynx indicates that trans- mission of virus by throat to-throat in fection did not play any major role Ac cording to Marine and coworkers [7] virus excretion from the pharynx is markedly reduced in parenterally vaccinated child ren Thi route of infection may thus be more common among pre-feeding non- v ccinated children

Summary

The pread of ttenuated vaccine virus Type I Chat train was studied in 13? families with _ or more children, mainly in the pre-school age One child in each family was fed live virus. Eleven of the 32 vaccinees who were 1 years old and o of 93 vaccinees between 3 and o years were found to transmit virus. In the total material studied in Sweden i.e collect d from this trial and from an earlier tudy in 20 families, the transmission of virus was found to be 10 times more frequent from children below _ years of age than from the 3–o year-old children

References

1. BÖTTI ER, M O AD H and LA ERCRA TZ, R.: Vaccination with ttenuated poliovirus Type 1 the Chat strain. I A study in ?0 families Acta Pædiat Scand 5 403 1966
2. BÖTTIGER, M KAISER, K and LUND- STRÖM, R Oral poliovaccination Nord Med 69 _09, 1963.
3. BÖTTIGER, M., ABBO, L., LUN ECK, H and N LERSTEDT C R.: Immune response to accination with inactiv ted poliovirus vaccine in S eden. Acta Path Microbiol Sc nd in press.
4. B ER, F and SCHÄR, M.: Poliom elitis accination with li polio virus. Am J Dis Child 101 566, 1961.
5. KOPROW KI H Discussion of Sabin A

Properties of attenuated polioviruses and their behavior in human beings. New York Acad Sc (Spec Publ.), 5 113, 1957
6. — Influence of age on susceptibility to infection with live attenuated poliovirus. Fifth Int Poliomyelitis Conf Copenhagen 1960, J B. Lippincott Co, p. 290.
7. MARINE, W M., CHIN, T D and GRAVELLE, C. R.: Limitation of fecal and pharyngeal poliovirus excretion in Salk vaccinated Children. Am J Hyg, 76 173, 196_
8. DU PAN R. M., PACCAUD M. and BERNHART F Effet de la dose rappel chez des nourrissons vaccinés par voie orale contre la poliomyélite. Ninth Symp Europ Assoc Poliomyelitis and allied Dis, Stockholm 1963, p. 161.
9. PLOTKIN S. A., KOPROWSKI, H., RICHARDSON S. N and STOKES, J Vaccination of families against poliomyelitis by feeding and by contact spread of live attenuated virus. Acta Paediat Scand, 49 551, 1960.
10. PRZESMYCKI, F DOBROWOLSKA, H., GIEDZIŃSKA, J OLAKOWSKI, T STANCZYK, B. and KARCZEWICZ, D Report on field trials with live attenuated poliomyelitis vaccine of Koprowski in Poland. Am J Hyg 71, 275, 1960.
11. SABIN A. B. and coworkers: Community wide use of oral poliomyelitis vaccine Am J Dis Child 101 548, 1960.
12. VONOSHILOWA, M. K. and coworkers Results of serologic and virologic investigation of children in connection with mass immunization with Sabin oral live poliomyelitis vaccine. Eighth Symp Europ Assoc Poliomyelitis and allied Dis, Prague 1961 p. _39
13. WESSLÉN T GARD S. and OLIN G Inactivation of poliomyelitis virus by formaldehyde. Arch Virusforsch, 7 1_5, 1957
14. WITT G Virologische und serologische Untersuchungen im Zusammenhang mit der Schluckimpfung gegen Poliomyelitis (Sabin Typ 1) in Schleswig Holstein 1962. Zbl Bakt Abt Orig 189 345, 1963.

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Vaccination with Attenuated Type 1 Poliovirus the Chat Strain

III Antibody Response and Spread of Virus in Schoolchildren

by MARGARETA BÖTTIGER ERIK BÖTTIGER and BO ZETTERBERG

Combined vaccinations with inactivated and live poliovirus vaccines were studied in Sweden during the years 1957 to 1960 [* 3 4] in families with children in the pre-school age

Certain trends in the immunization resulting from the combination of the two types of vaccines and in the behaviour of live attenuated poliovirus in the vaccinated individuals became evident The pre-school children responded well to a challenge with live virus aft r a primary immunization with inactivated vaccine Only at pre-feeding titers of 1 1250 or higher was a resistance to the virus challenge noted The reaction of the adults was less consistent failures occurring even among individuals with low r titers. Special interest was directed to the pread of the live vaccin viru within the family A marked reduction of the transmission of virus was found from children above _ years of age compared with the younger ones. The present investigation extended these studies to children of school age

The vaccination trials in schoolchildren were carried out in steps, first a detailed study in a boarding school for blind children and, second, an enlarged vaccination trial comprising six primary day schools.

In *the f rst part* the following factors were studied:

1 Presence of neutralizing antibodies after arious immunization schedules.
2. Susceptibility to oral vaccination.
3. Transmission of irus from orally ac cinated schoolchildren to their class mates.
4 Antibody status prior to and 7 months after the oral accination.

In *the second part* information was obtained about:

1 Vaccination of ordinary day-school chil dren on a larger scale

Materials and Methods

Inact vated vacc nes. In 1957 the accine used was produced by Eli Lilly Pharmaceu tics, USA, and in 1958 and later vaccines produced by the N tional Bacteriological Laboratory in Sweden were emplo ed [1] The general schedule for immunization was to administer 1 ml of accine subcutaneousl three times, the first two doses 6 weeks part and the third dose one year later

L ve vaccine This consisted exclus el of Type 1 the Chat strain. In the first part f this study the original irus provided by H

koprowski, the Wistar Institute Philadelphia, batch 10A11 was used [7] In the second part the first passage of this strain prepared by the N tional Bacteriological Laboratory in Sweden was introduced.

A dose of 6 $\log_{10}$ $TCID_{50}$ irus was given in about 10 ml of lemonade followed by a full glass of lemonade.

Virus isolat ons were performed in oller tubes containing primary monkey kidney tissue as described earlier [2].

Serological tests were carried out according to the immuno-inactivation technique described previously [1]. Blood specimens were collected by finger puncture; 0.3 ml of blood was immediately mixed with 1.2 ml of a heparin solution. The lowest serum dilution that could be tested was thus pproximately 1:10. Each dilution was tested in four t bes.

Part I Vaccinations in a School for Blind Children

Plan of the study The boarding school for blind children had 150 inmates aged 7–19 years. This is the only school of its kind in Sweden and thus the inmates come from all social classes and from all parts of Sweden. The three youngest age groups, 7–9 years old, altogether 63 children, lived in one wing, boys and girls apart, 6–8 children in each dormitory Play rooms and dining rooms were shared. Teaching was coeducational in 6 separate classes. The older inmates, 10–19 years old, lived in a separate building, one to four in each room, but otherwise under the same conditions. They were divided into 8 classes. Vaccinations with three inoculations of inactivated vaccine had started in 1957 mainly of the age groups born in the years 1945–50 The third injection was given in the spring of 1958. In the spring of 1959 some of the unvaccinated older and younger age groups were given two injections. Altogether there were 40 unvaccinated inmates when this study started in December 1959. The procedure was as follows.

Blood samples were drawn from all pupils and tested for neutralizing antibodies against poliovirus Type 1 Sero-negati e children and those not fully accinated with three inoculations were gi en inacti ated vaccine. All except 4 children, whose parents had refused any kind f vaccination, were sero-positive to Type 1 before the introduction of the vaccine virus.

In March 1960 live irus (6 $\log_{10}$ $TCID_{50}$ of the Chat strain) was fed to one or tw children in each class. Those orally vaccinated children, 11 girls and 9 boys, will be referred to as index" children. Excretion of irus was followed in weekly stool pecimens from all pupils for one month. Six weeks later all participating children were challenged with live vaccine. Stools were again collected from all pupils 1 2 and 4 weeks after feeding Seven months later i.e. in December 1960 and January 1961, new blood samples were drawn.

Results

Vaccination with nactivated virus

Fig. 1 shows the distribution of poliovirus Type 1 antibody titers among the children in December 1959 at the beginning of this study The data were divided into three groups. group A—titers of the unvaccinated children (39 individuals), group B—titers of those vaccinated twice in the pring of 1959 (38 individuals) and group C—titers of children vaccinated twice in 1957 and once in 1958 (53 individuals) The age distribution of the children was approximately the same in the different groups. A successive reduction of seronegatives (titers < 10) and increase of antibody titers was shown to be related to the number of injections

Vaccinations with live vaccine

Antibody response. One hundred paired sera were obtained 4 months before feeding and 7 months after feeding the

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Combined vaccinations with inactivated and live poliovirus vaccines were studied in Sweden during the years 1957 to 1960 [3 4] in families with children in the pre-school age

Certain trends in the immunization resulting from the combination of the two types of vaccines and in the behaviour of live attenuated poliovirus in the vaccinated individuals became evident. The pre-school children responded well to a challenge with live virus after a primary immunization with inactivated vaccine Only at pre-feeding titers of 1 1 ..0 or higher was a resistance to the virus challenge noted The reaction of the adults was less consistent failures occurring even among individual with lower titers. Special interest was directed to the spread of the live vaccine virus within the family A marked reduction of the transmission of virus was found from children above - years of age compared with the younger ones. The present investigation extended these studies to children of school age.

The vaccination trials in schoolchildren were carried out in 2 steps first a detailed study in a boarding school for blind children and second an enlarged vaccination trial comprising six primary day schools.

In *the first part* the following factors were studied:

1 Presence of neutralizing antibodies after various immunization schedules.
 Susceptibility to oral vaccination.
3. Transmission of virus from orally vaccinated schoolchildren to their classmates.
4 Antibody status prior to and 7 months after the oral vaccination.

In *the second part* information was obtained about

1 Vaccination of ordinary day-school children on a larger scale

Materials and Methods

Inactivated vaccine In 1957 the vaccine used was produced by Eli Lilly Pharmaceutics, USA, and in 1958 and later vaccines produced by the National Bacteriological Laboratory in Sweden were employed [1]. The general schedule for immunization was to administer 1 ml of vaccine subcutaneously three times, the first two doses 6 weeks apart and the third dose one year later

Live vaccine This consisted exclusively of Type 1 the Chat strain. In the first part of this study the original virus provided by H

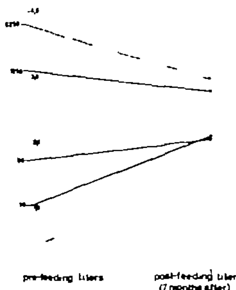


Fig. 3. Post feeding antibody titers (geometric mean level) of groups with different pre-feeding antibody levels (blind schoolchildren).

Virus excretion. In 19 of the 20 index children the virus was isolated from the stools (Table 1). The second vaccination with live virus (Table) 6 weeks later included the original 20 index children and all others except 22 children. Virus was isolated from 90 per cent and 60 per cent respectively of the vaccinated children in the four lower and higher grades. Of the 20 orally revaccinated index children 13 had negative stool specimens. Four of 7 virus excretors, however might still have been excreting virus after the first feeding.

TABLE 1. *Feces isolations after feed g of the index children*

	Excretors	Non excretors
Index children	19	1
Contacts	3	118

TABLE . *Feces isolations after the general feeding*

	Excretors	Non-excretors
Index children and earlier infected contacts	9 (−4)[a]	13
Other acolnees		
age 12 19 (n = 33)	20 (61 %)	13 (39 %)
age 7–11 (= 57)	51 (89 %)	6 (11 %)
Contacts	(1)[a]	15

These children might still excrete irus after the first feeding.

as their last feces specimen collected before the re-vaccination still contained virus.

In the first introductory study [2, 5] it was indicated that an inverse correlation existed between the antibody titers at the time of feeding of virus and the duration of the virus excretion. In order to elucidate these circumstances the individuals were divided into two groups:

Group 1. Resistant children whose fecal specimens were negative or positive for only one week after feeding.

Group *. *Susceptible children* excreting virus for weeks or more.

Group 1 (22 individuals) had a geometric mean antibody titer of 1 1..0 before feeding and group * (5.. individuals) a mean titer of 1 75. In Fig. 4 the children are divided into groups according to pre-feeding titers and the numbers of children resistant and susceptible to virus challenge are shown. The successive reduction of long-time excretors with increasing pre-feeding antibody titer is clearly demonstrated.

Spread of virus. At the first feeding the 19 children who excreted virus could be regarded as potential spreaders of virus.

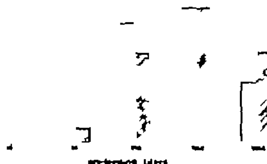


Fig. 4. Correlation between pre-feeding antibody titers and virus excretion. White columns represent number of children excreting virus for 2 weeks or more and black columns non-excretors or children excreting for one week only

Feces samples were obtained from 110 contacts. Three of these became infected, two children in the first and one child in the second grade. The latter had refused any kind of vaccination and was initially sero-negative (Table _)

After the second general feeding feces samples were obtained from 15 of the 22 children who had refused live vaccine. No evidence of viral infection in these children appeared.

Untoward reactions. There were no untoward reactions that could be attributed to vaccination.

Part II. Vaccination in Six Primary Day Schools

Plan of the study. This part of the vaccination trial was carried out in the spring of 1961 in six ordinary primary day-schools comprising close on 5000 children, aged 7 to 15 years. The schools were all located in a fairly densely populated suburban area north of Stockholm. The majority of the families belonged to the middle social class.

To enter the study it was a prerequisite that the child and its family had received at least two inoculations of inactivated vaccine. Members of families born 50 years of age were exempted from this rule as, according to earlier investigations [9], they were more likely to be naturally immune to poliovirus infection.

Of the 4823 children in the area studied the parents of 4212 consented to their children participating in the oral vaccination. The vaccination status of about 20 000 persons was checked and close on 8300 doses of parenteral vaccine were administered.

The oral vaccine was distributed between March 20 and 23, 1961. It was administered by adding one drop containing approxi

mately 6 $\log_{10}$ $TCID_{50}$ of virus into a paper beaker and diluting with 10–20 ml of lemonade. Altogether 3500 children participated. *Almost 15 per cent of the children* whose parents had consented to oral vaccination were absent due to illness, as in all the schools an infection causing vomiting and diarrhoea had started a few days prior to the vaccination.

Serological and virological investigations were carried out in about 100 children in the first grade. Blood samples (0.1 ml) taken by finger puncture and diluted in 1.2 ml of heparin solution were collected prior to and 2 months after the feeding of live virus. Feces samples were also collected from a number of the children, one before and the other within two weeks after the oral vaccination.

The general health of the schoolchildren was followed during the subsequent 3 months by the schools health centers, assisted by special medical personnel.

A general survey of the epidemiologic condition in the area was carried out during the following year

Results

Antibodies in children in the first grade. The antibody status before and 2

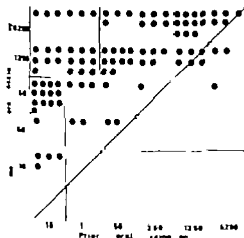


Fig. 5. Correlation between pre- and post-feeding titers in individual children.

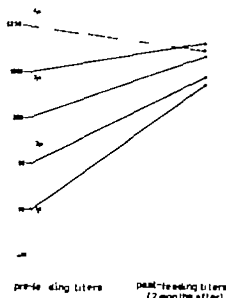


Fig. 6. Post-feeding antibody titers (geometric mean level) of groups with different pre-feeding levels (ordinary schoolchildren).

months after the distribution of live vaccine to 100 children in the first grade is illustrated in Figs. 5 and 6. In Fig 5 the pre-vaccination titers are plotted against the post-vaccination values. All children tested were found to have demonstrable antibodies in the dilution 1 10 two months after feeding, whereas almost one-third were negative *at this serum dilution before* oral vaccination. Ninety-six per cent had titers of 1 50 or higher Fig. 6 illustrates the mean antibody values of the different pre-feeding groups 2 months after feeding Serum was not diluted beyond 1 62.0. A general equalization of titers irrespective of pre-feeding values is indicated.

Excretion and spread of virus About 80 per cent of the children in the classes investigated were orally vaccinated. Fecal samples, one collected prior to and one

collected 1 or 2 weeks after feeding of live virus were obtained from 135 vaccinees In three of the pre-feeding samples adenovirus was found. From 124 of the post feeding samples (92 per cent) poliovirus was isolated. From 8 of 11 vaccinees with negative tool samples pre-feeding blood samples demonstrated antibody titers of 1 30 or above Twentytwo fecal samples from unvaccinated classmates were all negative

Untoward reactions Children falling ill during the weeks after vaccination were physically examined. No symptoms of paralysis appeared, nor any other untoward reactions that might be attributed with certainty to the vaccination. However one case with an unclear allergic manifestation of facial oedema and fever appeared one week after the feeding During the following year no case of paralytic poliomyelitis was registered from the area. No systematic investigation was made of the spread of the vaccine virus within the area but no positive poliovirus isolation from the between 500 and 1000 virus isolations carried out during the following year was reported from the Stockholm area.

Discussion

The school for blind children was chosen for the first investigation of the effect and spread of live poliovirus vaccine in schoolchildren. These children provided maximal possibilities for dissemination of enteric viruses as their handicap imposes particular hygienic difficulties on them and their mode of intercommunication is to a large extent by touch.

Antibody response to vaccination with inactivated poliovirus The preceding vaccinations with inactivated vaccine had greatly reduced the number of seronegatives However one-and-a-half year after three injections given in 1957 and 1958, 25 per cent of the children had no demonstrable antibodies to Type 1 at a dilution of 1 10. Among the unvaccinated 60 per cent were negative.

Antibody response to live vaccine

Ninety per cent of the blind schoolchildren with pre-vaccination antibody titers lower than 1 50 showed persisting titer rises 7 months after feeding. Among the day-school children 4 of 29 vaccinees with pre-vaccination titers less than 1 10 did not exhibit titers above 1 10 two months after feeding. This indicated that the majority but not all, of the schoolchildren with low pre-feeding antibody levels reacted with an active antibody response to stimulation with live poliovirus antigen.

As was found from earlier studies by the authors [3 4], vaccination resulted in a general equalization of titers irrespective of pre-feeding antibody levels. This tendency was slightly less marked however among the schoolchildren than among the younger age groups earlier investigated.

Excretion of vaccine virus

The live virus vaccination was performed in the winter and spring, a time of the year when generally few interfering viruses circulate Ninety per cent of the younger and 60 per cent of the older blind schoolchildren excreted virus following vaccination. (The mean pre-vaccination antibody titers of the non-excretors exceeded 1 1-50.)

Although only one fecal sample was collected from the day-school vaccinees, the same proportion of excretors (90 per cent) was found as among the blind schoolchildren of the same age, who were tested weekly for four weeks

Spread of virus

Earlier studies had indicated that contact infections from vaccinees older than years were relatively rare

A study similar to the present one was carried out by Przesmycki *et al.* [11] in Poland with the Chat strain. In a boarding school for boys, where all had been pre-vaccinated with inactivated vaccine 3:. of the 7 boys were given oral vaccine. Among their 30 unvaccinated school fellows virus was isolated from the stools of u.

The authors study in the boarding school indicated that there was very little tendency to dissemination of the Chat strain among these schoolchildren. Despite the children s particular handicap only 3 of at least 70 contacts presumably susceptible to virus infection (as judged from the results of the second general feeding) were found to excrete virus. These three all belonged to the youngest age group. After the second general feeding when 80 of 1L. vaccinated children ex creted virus, no spread of virus was found to the 15 unvaccinated inmates studied. Half of these unvaccinated controls had no detectable or low antibody titers to poliovirus Type 1

There was no sign of transmission of vaccine virus within the ordinary day school. Although 80 per cent of the child ren were fed live virus vaccine and t least 90 per cent of these excreted virus, none of the unvaccinated children tested excreted virus. This lack of spread was also con firmed with Chat vaccine produced in human diploid cell strains [10].

Untoward reactions

No increased morbidity of minor ill-nesses of different kinds that could be connected with the vaccination was observed. From 3 blind children with a sore throat in the week after v ccination hemolytic streptococci were isolated.

The gastro-enteric disease that occurred prior to and after the feeding of the or dinary day school children did not appear to be influenced by the vaccination or vice versa.

Safety

When the large-scale oral vaccinations in the day-school were undertaken, there were still a large percentage of unprotected adults among the population. The risks of introducing a live poliovirus into such a population could not be disregar ded. Thus, as a precaution unvaccina ted family contacts were inoculated with the inactivated v ccine. No paralytic cases ppeared.

The material is too small for estimation of the safety of the vaccine however That it is safe has been confirmed by large-scale vaccinations in other countries [8].

General remarks

Extensive studies have been made of vaccination with live poliovirus vaccines. Many of the results have been diverging. One might assume that both the specific genetic characters of the viruses and the particular circumstances in the popula tion and region in which they are intro-

duced might influence the behavior of the virus. Regardless of whether a dissemination of virus is regarded as advantageous or not, the behavior of the particular vaccine strain should preferably be studied in a community similar to that into which it is intended to be introduced. According to the experience gained in this and earlier studies [2, 4] the rate of spread of the Chat vaccine was limited in the Swedish community and was found to be inversely correlated to the age of the vaccinee. Forty three per cent of the vaccinees below 2 years of age, 3 per cent of the 4-year-olds and none of the ordinary schoolchildren were found to transmit virus.

Also the antibody response appeared to some extent to be age-dependent. All the pre-schoolchildren tested had titers of 1:50 or above after the feeding. Several schoolchildren and adults were found however whose antibody titers remained at about 1:10 although they had excreted virus after vaccination.

Summary

Immunization with live attenuated poliovirus Type 1 the Chat strain, was performed in schoolchildren after previous vaccination with inactivated poliovirus vaccine.

The results indicated that the majority but not all of the schoolchildren with low pre-feeding antibody titers (≤1:10) reacted with antibody stimulation to feeding with live attenuated vaccine. These results differed from those experienced with pre-school children. All the latter had titers of 1:50 or higher after vaccination with the live attenuated virus.

No spread of vaccine virus was observed among the ordinary day-school children. Among the blind boarding-school children the spread appeared very limited, although their particular handicap favours transmission of virus by touch. When 3–10 per cent of the inmates were fed live vaccine, 3 of at least 40 susceptible contacts became infected. These 3 children belonged to the 2 youngest grades.

References

1. Böttiger, M., Abbo, L., Lundbeck, H. and Salenstedt, C. R.: The immune response to vaccination with inactivated poliovirus vaccine. *Acta Path Microbiol Scand* in press.
2. Böttiger, M., Gard, S. and Lagercrantz, R.: Vaccination with attenuated poliovirus Type 1, the Chat strain. I. A study in 70 families. *Acta Paediat Scand*, *45*: 403, 1956.
3. — Vaccination with attenuated live poliovirus. *Acta Path Microbiol Scand*, Suppl. 144, *51*: 303, 1961.
4. Böttiger, M., Gard, S. and Zetterberg, B.: Vaccination with attenuated Type 1 poliovirus, the Chat strain. II. Transmission of virus in relation to age. *Acta Paediat Scand*, *45*: 416, 1956.
5. Gard, S., Böttiger, M. and Lagercrantz, R.: Vaccination with attenuated poliovirus Type 1, the Chat strain. *First Int Conf Live Poliovirus Vaccines*, *Pan Am San Bur* Sc. Publ. No. *44*: 350, 1959.
6. Gard, S.: Field and laboratory experiences with the Chat strain, Type 1 poliovirus. *Second Int Conf Live Poliovirus Vaccines*, *Pan Am San Bur* Sc. Publ. No. *50*: 187, 1960.
7. Koprowski, H.: Discussion of Sabin A.: Properties of attenuated polioviruses and their behavior in human beings. *New York Acad Sc* (Spec Publ.) *5*: 113, 1957.
8. Kostrzewski, J., Kulesza, A. and Zieleska, H.: Vaccination against poliomyelitis in Poland with Types 1 and 3 attenuated viruses of Koprowski. — Epidemiological evaluation of results of mass vaccination 1959–60. *Bull Wld Hlth Org*, *26*: 745, 1962.
9. Olin, G. and Wesslén, T.: Seroimmune patterns for poliomyelitis in Sweden. *Arch Virusforsch*, *7*: 191, 1957.
10. Pagano, J. S., Böttiger, M., Bonnevier, J. O. and Gard, S.: The response and lack of spread in Swedish schoolchildren given

an *attenuated poliovirus vaccine prepared* in human diploid cell strain. *Am J Hyg* 79 74, 1964.
11 PRZESMYCKI, F DOBROWOLSKA, H., GRO CHADOW, L., OLAKOWSKI, T STANCZYK, R. and MARUSZEWICZ, P Report on field trials with the attenuated poliomyelitis vaccine of Koprowski in Poland *Bull Wld Hlth Org* 26 745, 196_

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CASE REPORT

Weber Christian Syndrome in a Newborn

by S. VESTERMARK

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The Weber Christian syndrome or nodular nonsuppurative panniculitis, is a fairly infrequent disease which occurs mainly in adult females [1]. The condition is characterized by subcutaneous nodules which, in typical cases, are accompanied by fever, fatigue, poor general condition and sometimes also by pains in joints and muscles [4]. Occasionally enlargement of liver and spleen has been observed in children [8, 11, 12, 13]. The subcutaneous nodules, which will generally leave atrophic scars, may recur but occasionally years may pass between the episodes of recurrence [3].

The disease may occur at any age although only a few cases have been described in children. Up to 1945 a total of 6 cases had been reported [4]. By 1957 this figure had risen to 12 [8] and in 1960 Wattiez *et al.* [16] stated that about 20 cases had been described in children. More recently a few cases have been added to the list [13]. Most of the cases described in children have begun at the age of 8 to 10 years, and only in a few cases have the symptoms set in as early as 6 months of age [1...].

Herein we report a case in which the subcutaneous nodules were present from birth.

Case Report

The patient, a female, was the youngest of five siblings, one of whom died of pneumonia at the age of 8 months. The mother was perfectly well throughout the pregnancy and did not receive any drugs. Delivery was normal. Birthweight was 3,400 g. From birth she had two large tender nodules in the natal region. At six weeks of age she was admitted to this hospital with pneumonia. On admission there was some thrush which disappeared gradually within 3 weeks after treatment with mycostatin and swabbing with silver nitrat. On either side of the anal cleft two firm, well-defined, smooth, tender nodules could be seen and palpated. The nodules measured 5 cm by 6 and were approximately 1 cm deep. The nodules could be moved freely in relation to skin and the underlying tissues. The skin over the nodules was moderately red. On the left upper arm several small, firm, tender, smooth, well-defined nodules were felt freely movable in relation to the skin and the underlying tissues. In this region the skin over the nodules was of normal colour. The nodules disappeared gradually—those on the left upper arm in the course of 4 weeks and in the natal region in the course of 6 weeks. The skin on the dorsum and the legs was firm and somewhat thickened but without any nodules. The colour was normal. During her stay in this hospital the liver increased in size and could be felt 3 fingerbreadths below the right costal margin when the patient was discharged at the age of 3 months. The temperature was normal during the entire stay in hospital

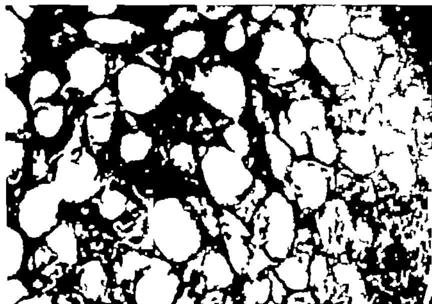


Fig. 1 Biopsy from a subcutaneous nodule. Fatty tissue with fibrillary connective tissue foam cells, giant cells, and lymphocyte infiltration; lipophagic stage (hematoxylin and eosin)

Laboratory studies. Sedimentation rate 65–85 mm/hr White cell counts: 18,500–30,200–13,850. Differential count: 37 granulocytes, 50 lymphocytes, 6 eosinophils, and 7 mononuclear leukocytes. Cold agglutinin titer and adenovirus titer normal. Antistreptolysin titer antistreptococcal hyaluronidase titer and antistaphylosin titer normal. Serum protein electrophoresis and serum lipid fractions normal. Anti-globulin consumption test normal. Cryoglobulin not demonstrable. Immuno-electrophoretic studies were normal apart increase in alpha-2 M-globulins. Urinalysis was normal, as was a chest X ray Culti ation f tissue from one nodule revealed a multitude of gram-positive cocci, growing with yellow colonies, but without fermenting mannite or coagulating plasma. Pharyngeal swab showed candida albicans, but no actinomyces.

Histological examination of biopsy from a nodule on the buttocks showed the following picture (Fig. 1). Fatty tissue surrounded by patches of fibrillary connective tissue. In the fatty tissue several phagocytic histiocytes, foam cells and a few giant cells were seen. There was slight extra asation of blood into the f tty tissue and in some areas slight infiltration of lymphocytes and polymorphnuclear granulocytes. At the border of the tissue sample normal sebaceous glands were seen. No signs of malignancy and no fatty acid crystals. Histological examination of skin biopsy from the thigh (Fig. 2) showed pronounced hyaline fibrosis in the corium and the upper layers of the subcutaneous connective tissue. The sebaceous glands and the sweat glands were somewhat atrophic, presumably because of the pronounced fibrosis. In the subcutaneous tissue a few vessels with thickened walls were seen, the thickening was caused to a certain extent by muscular hyperplasia, but in particular by fibrosis.

The patient was treated with penicillin for 10 days. At a follow up examination when 6 months old the patient was perfectly well. Her development was normal. There were no nodules and the skin ppeared normal. At the sites of the former nodules on

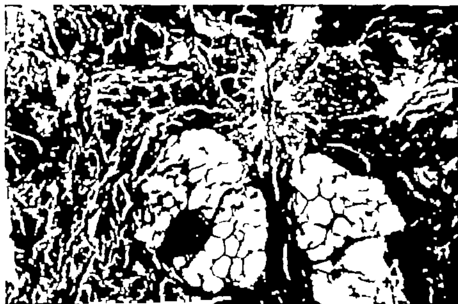


Fig. 2. Biopsy from the skin. Subcutaneous tissue with fatty tissue surrounded by pronounced hyaline fibrosis fibroblastic stage (Gleson).

the buttocks small scars were seen. The en largement of the liver was unchanged. The sedimentation rat was still increased (09 mm/hr). The leukocyte count was 29,300 with slight lymphocytosis. SGO transami nase was increased (3.10 mmol/l/hr —normal range 0.35–1.35). Electrophoresis of lactic ydrogenase showed fractions 3, 4 and 5 be increased, as seen in li er diseases.

Discussion

Our patient had no fever and the scars left by the subcutaneous nodules were very slight. However the clinical picture, the increased sedimentation rate and the hepatic enlargement make it justifiable to classify the case as Weber Christian syndrome. This classification is verified by the histological appearance of one of the nodules. It is well known that the syndrome will not always present the typical picture, since one or more of the symptoms may be absent. In some cases the general condition will be unaffected and there will be no fever [–, 15], as also the scar formation may fail to occur [11]. Hence the clinical picture is not always well-defined and no doubt transitional forms to the Rothmann Makai syndrome may exist [7 14]. In the Rothmann Makai syndrome the nodules develop very rapidly and disappear quickly without leaving scars. The general condition is unaffected and there is no fever [4]. Both the Rothmann Makai syndrome and the Weber-Christian syndrome may present histologically three stages: the first is the inflammatory the second the lipophagic and the third the fibroblastic stage [1 0], but it is not possible to distinguish between the two syndromes histologically [3 4]. Hence, it has been suggested that the Rothmann Makai syndrome might be a variant of the Weber-Christian syndrome [6] In our patient the histological ap-

pearance of the biopsy from the subcutaneous nodule corresponded to the second stage, whereas the later skin biopsy corresponded to the third stage. It will hardly be difficult to differentiate Weber-Christian syndrome from other subcutaneous nodules as e.g. erythema nodosum, erythema induratum and lipomatosis. Furthermore, adiponecrosis subcutanea neonatorum must be born in mind in infants, but this condition is not accompanied by affection of the general condition and the microscopic picture is different. Disseminated lipogranulomatosis or Farber's disease has only rarely been described at birth. The initial symptoms are vomiting, hoarseness and periarticular swellings and later subcutaneous granulomas [16] but the granulomas are "non relapsing".

Presumably the etiology is multifarious [1, 2, 4, 12]. In a few cases pathogenic bacteria have been demonstrated at biopsy [12] and sometimes the nodules develop in connection with an infection [10, 11] but in most cases the etiology is unknown. In our patient gram positive cocci were demonstrated at biopsy but these cocci were not able to coagulate plasma and are, therefore, unlikely to be pathogenic. However, the persistently increased sedimentation rate and the enlarged liver show that the patient still suffers from a generalized disorder and that the nodules are but a secondary manifestation of this disease.

The prognosis will depend mainly on the underlying disorder but in the majority of cases the prognosis is fair. However Sanford *et al.* [1.] reported a fatal outcome in an 8½ month-old boy. In several fatal cases in adults autopsy has shown that the liver and the visceral fatty tissues may be affected too [6]. The prognosis in our patient is uncertain. True enough she thrives and is now quite unaffected, but her liver is still enlarged and her sedimentation rate significantly raised.

Because of the obscure and presumably multifarious etiology, treatment must be purely experimental. Indeed, numerous drugs such as antibiotics, steroids, antimalarial agents, antihistamines and antirheumatics have been tried with varying success during the years [5]. On the assumption that our patient might suffer from an infection, she was treated with penicillin. No effect of this therapy was observed. Her sedimentation rate did not change and the subcutaneous nodules disappeared gradually without any relation to the treatment.

Summary

A case of Weber-Christian syndrome in a newborn girl is reported. The subcutaneous nodules were localized to the left upper arm and the buttocks. Her general condition was affected, but there was no fever. The nodules disappeared at the age of 3 months leaving small scars, and her general condition improved gradually. At the age of 6 months, however, her sedimentation rate was still increased, there was leukocytosis and the liver was enlarged. Biopsy from one of the subcutaneous nodules showed histological changes corresponding to the second stage. The etiology is unknown.

Ref rences

1 ALBRECTSEN B The W ber-Christian y drome with particular reference t th kgy Acta Dermatovener (Stockholm), 40 474 1960.

2 AR LD H. A. and BAINBOROU H, A. R.: W ber-Christian disease with visceral in olvement. Case report and review of the literature Canad Med Ass J 89 1128 1963.

3. BAUMANN R.: Die Pannikulitisformen unte besonderer Berücksichtigung der Spontan pannikulitis Rothmann Makai. Ärztl Wschr 8 609, 1953.

4. B UMGARTNER, W and RIVA, G Panni kulitis, die herdförmige Fettgew bsent zündung Hel Med Acta, Suppl. 14 1 1945.

5. BENSON, R and FOWLER, P D Treatment of Weber-Christian disease Brit Med J II 615, 1964.

6. COCHRANE, G M Weber-Christian disease with visceral i vol ement. Brit Med J II 281 1958.

7 DUPERRAT M B Panniculite de Rothmann Makai. Med Infant (Paris), 67 5, 1960.

8. GILLET P BARTMAN and ACHTEN G Panniculit nodulaire recidivant febrile (ou maladie de W ber-Christian). Arch Franc Pediat 14 768, 1957

9 LAYMON C. W and PETERSON, W C., JR.: Lipogranulomatosis subcutanea (Roth mann-Makai). Arch Derm (Chicago), 90 288, 1964

10. MIEDZINSKI, F Some remarks about Weber-Christian disease Acta Dermatovener (Stockholm), 37 88, 1957

11 ROGET J C UDERC J BEAUDO G, A. and GUILBOT J Un cas de panniculit nodulaire récidi ant fébrile. Pediatrie, 12 191 1957

12. SANFORD H N EL ANE, D F and STEIN F Chronic panniculitis with leu openia (Weber-Christian yndrome). Ame J Dis Child 83. 156, 195_

13. STRZELECKA, M Przypadek panniculitis nodularis non suppurativa febrilis recidivans. Pediat Pol, 36: 639 1961

14. TURPIN R. and BOCQUET L.: Panniculite aigue bénigne Sem H p Paris, 24. 1793, 1948.

15. W TTIER, R., ACHTEN G and VOUSSURE, G La maladie de W ber-Christian. Une observ tion lunique atypique Acta Paediat Belg 14 223, 1960.

16. ZETTERSTRÖM R.: Disseminated lipogranulomatosis (Farber disease). Acta Paediat Scand 47 501 1958.

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CASE REPORT

Poisoning in Children Induced by Trichlorisobutylalcohol Suppositories

by P. KEMÉNY and E. CSONTOS

From the Children Hospital, Madarasz-Street Council of XIII District, Budapest, Hungary

Drug poisoning in childhood constitutes an increasingly complex problem, as the list of drugs known to cause toxicity rises. In particular drugs causing central nervous system toxic effects pose acute difficulties because of the non-specific nature of many of the signs and symptoms.

In Hungary suppositories containing trichlorisobutylalcohol (Nautisan®) are frequently administered to counteract vomiting. The adult suppository contains 1.0 g trichlorisobutylalcohol and 0.1 g caffeine, while the paediatric preparation contains 0.05 g trichlorisobutylalcohol and 0.015 g caffeine.

We have recently seen three children in whom poisoning by this drug could be implicated. Since, to our knowledge toxicity from trichlorisobutylalcohol has not previously been reported except in Hungarian publications, we wish to draw the attention of physicians to its potential danger when absorbed in high dosage.

Case Reports

Case 1

A 7½ year old girl was admitted to the hospital with a 4 day history of high fever and repeated vomiting. On examination she was acutely ill and moderately dehydrated. The major findings were in the central nervous system. She was very drowsy, fell asleep repeatedly while being examined and was incontinent. Though her deep tendon reflexes were normal, superficial reflexes were absent. There was generalized muscle hypotonicity. When wakened, she responded to questions appropriately however she was markedly dysarthric. A coarse horizontal nystagmus was noted on lateral deviation of the eyes, and an equally coarse vertical nystagmus when upward gaze was elicited. However the remainder of the eye examination was normal, as was fundoscopic inspection. There was no evidence of meningeal irritation, and the cerebrospinal fluid was normal. Apart from inflammation of the nasopharynx, the remainder of the physical examination was normal.

Careful questioning of the parents revealed that the mother had administered 5 adult Nautisan suppositories to her daughter during the preceding 3 days because of the vomiting. It was then realized that the drowsiness and other central nervous system signs might well be due to poisoning by the drug rather than simply secondary to dehydration as had been at first thought.

The dehydration was managed with an intravenous infusion of equal parts Ringer solution and 5% glucose. After 24 hours the vomiting finally ceased, however her marked somnolence and other CNS signs persisted for the first 3 days, after which they gradually remitted. By the end of the week recovery was complete.

Case

An 8 year old boy was admitted with an 8 day history of fever and vomiting with increasing drowsiness over the 48–72 hours immediately preceding admission. The suggested diagnosis was encephalitis. On examination he was acutely ill and comatous, responding only to painful stimuli. As in case 1 the prominent findings were in the central nervous system. There was a generalized muscular hypotonus. Superficial reflexes were absent while the deep tendon reflexes were present. Like Case 1 he had a coarse horizontal and vertical nystagmus in the presence of an otherwise normal eye examination. There was no evidence of meningeal irritation and the cerebrospinal fluid was normal. The rest of the physical examination was non-contributory apart from evidence of an upper respiratory infection.

History was obtained from the parents of the administration of 10 adult Nautisan suppositories over the 5 days preceding admission.

An intravenous infusion was commenced on admission. Despite this, his course over the first 72 hours in hospital was stormy with a further deterioration in his state of consciousness on the second and third day at which time it was felt desirable to administer CNS stimulants parenterally.

From the 4th day on a steady improvement in his condition ensued with a constant gradual regression of the abnormal central nervous system signs. By the 9th day recovery was complete.

Case 3

A 14 month old boy weighing 10 kg was admitted because of the accidental absorption of ad lt Nautisan suppositories. Within 12 hours somnolence and hypotonicity ensued. Nystagmus was not observed. The abnormal signs persisted for 24 hours following which gradual recovery occurred. No specific therapy was given during the hospital stay.

Discussion

In all these cases, absorption of high doses of trichlorisobutylalcohol was followed by evidence of central nervous system affection the signs generally being of the non-specific nature typical of many drug poisonings and central nervous system disorders. However in addition we observed horizontal and vertical nystagmus in the 2 older children, and we believe this may be peculiar to poisoning by this particular drug. Since antiemetics are frequently used in paediatric practice we believe that the potential dangers of excessive dosage of these drugs should be emphasized, and we present this case report as an example of 3 such cases in which trichlorisobutylalcohol was the offending agent.

Summary

Three cases of trichlorisobutylalcohol poisoning in children are reported. While non-specific central nervous system changes were prominent the horizontal and vertical nystagmus observed was considered to be characteristic to poisoning with this drug. The importance of caution in the use of antiemetics in paediatric practice is stressed.

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BOOK REVIEWS

Ulrich Keuth: Das Membransyndrom des Früh- und Neugeborenen.

Experimentelle Medizin, Pathologie und Klinik, Band 16 Springer Verlag, Berlin, Heidelberg and New York. 153 pages, ill. Price DM 36.

Hyaline membrane disease is a very common cause of death in the newborn and has been the subject of many investigations in the last years. The author gives a synopsis on the problem discussing its etiology, pathology, clinical picture, prophylaxis and therapy. The many different theories concerning genesis of the syndrome are discussed and it is stressed that the disorder should be looked upon as a respiratory stress syndrome more than a specific disease. The bibliography includes more than 600 references making the book especially valuable not only to pediatricians in general but also for research purposes. It remains to be seen if more recent suggestions to rename the syndrome ("pulmonary hypofusion syndrome") will be generally accepted.

O. Kretschmer

Basil Kiernander (ed.). Physical Medicine in Paediatrics.

Butterworth & Co. Ltd., London, 1965. 231 pages, ill. Price 65 s.

This volume is a welcome contribution to better understanding of all the complicated problems around a handicapped child. Physical medicine in the sense it is used in the book goes beyond physical therapy and includes e.g. electrodiagnosis of muscular hypertonia in infancy and childhood which is dealt with in an excellent review. Chapters on growth, physical fitness in children and human posture serve as an introduction to subsequent parts on the treatment of cerebral palsy, orthopaedic disorders and rheumatic diseases. Some repetitions have been unavoidable and are not too disturbing. One is, however, looking in vain for some principles or practical advice concerning the care of children with spina bifida and myelomeningocele, a complicated problem of increasing importance. The quality of some of the illustrations should also be improved in a coming edition.

B. Hellström

J. Apley and R. MacKeith: Das Kind und seine Symptome in psykosomatischer Sicht.

Hippokrates-Verlag Stuttgart, 1965, 300 pages. Price DM 32.

Translated into the German language by Ruth Hafter-Gass. First English edition 1962.

The book deals with the child and his symptoms originating from organic and emotional disturbances. For every symptom, possible organic diseases underlying it are shortly reviewed after which the main discussion concerns the child's psychosomatic, emotional and social problems. The importance of evaluating the child's whole personality and character rather than concentrating only on symptoms is stressed. The doctor's advice and especially the manner in which it should be given to the parents is discussed in detail. This last aspect cannot be overemphasized in the age of large impersonal urban communities. The book is of great interest for pediatricians, practitioners and medical students being a counterbalance to the rapidly increasing amount of books on molecular medicine.

Klas Thorell, Stockholm

Philip Rubin: Dynamic classification of bone dysplasias

Year Book Publ., Chicago, 1964. 410 pages, ill. Price $ 27

The field of bone dysplasias comprises many items, of which quite a number occur very infrequently. Accordingly the experience of an individual clinician or research worker in this very broad field will be

rather limited. A modern comprehensive presentation therefore fills a great need. In his book Dr Rubin gives us a broad introduction to the field with a good review of the essentials of the present knowledge of bone morphology from the macroanatomic level down to the molecular level. He also gives us a more detailed account of bone remodelling under normal conditions and of experimental modification under various abnormal influences. These data are used as a basis by the author to present us with a classification of bone dysplasias in which the main site of the modelling error is the common denominator. According to this classification the dysplasias are grouped into those involving the epiphysis, physis, metaphysis and diaphysis; Hypoplastic or hyperplastic types are to be found in each group. In my opinion, the proposed classification of bone dysplasias has many advantages, apart from its simplicity and I believe that it will be widely used. With our present knowledge it is, however, evident that there are several bone dysplasias which are not easily included in the system.

I think it is important to stress one point put forward by Dr Rubin, namely that many of these diseases occur so infrequently that some sort of centralized study of them would be of value. In order to gain insight into the pattern of these cases, it is of fundamental importance to follow them up for many years using X rays, metabolic studies and biopsies. At present we know very little about the underlying mechanism for many of these dysplasias, and it is to be expected that genetic and biochemical studies will be of fundamental importance to the progress in this field.

The different bone dysplasias, arranged according to the above mentioned principles, are discussed separately. Here Dr Rubin gives an excellent review of these conditions from the clinical, radiographical and morphological viewpoints, and the list of valuable references will be of great service to people interested in going deeper into the problems. The presentation is clear and the illustrations are, in general, of a high standard.

The book gives us a complete and up to date review of the complex field of bone dysplasias, and can be recommended to clinicians and radiologists and also to people working in the corresponding basic sciences.

Bengt Engfeldt

ANNOUNCEMENT

The World Health Organization Requires Specialists in Maternal and Child Health

Applications are invited for posts of medical officers in Maternal and Child Health at Headquarters and/or Regional Offices of WHO. Applicants must be graduates of approved medical schools and have postgraduate training in paediatrics and/or obstetrics. A diploma in public health or equivalent is desirable. Applicants should be experienced in general public health work, particularly in the administration of maternal and child health services. They should also have had progressively responsible experience of a supervisory and advisory nature.

Persons who meet these requirements are invited to write for an application form to:

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Geneva, Switzerland

Acta Paediatrica Scandinavica 55. 441–451 Sept. 1966

From the Department of Pediatrics, School of Medicine University of California, Los Angeles, California, U.S.A.

Effect of Lung Expansion on the Fetal Lamb Circulation

by GÖRAN ENHÖRNING [1] FORREST H ADAMS and ANNE NORMAN

In 1953 Dawes and co-workers [9] reported that if the lungs of fetal lambs were expanded with air oxygen or nitrogen, the resistance to pulmonary blood flow was greatly reduced. If on the other hand, the lungs were expanded with saline solution the resistance was not significantly influenced. These findings suggest that surface tension might be an important factor in causing the increase in pulmonary blood flow which characteristically occurs just after birth.

During the last seven years there has been an increasing interest in the problems of pulmonary surface tension. The results obtained with two different methods [5, 20] have suggested that surface tension in the air liquid interface of the alveoli is very low It thus seems quite unlikely that surface tension would have any great influence on the pulmonary circulation. Using a new method for determining surface tension [13 14 15] we have come to the conclusion that at least in the immediate postnatal period surface tension is probably not as low as later in life and could thus be of importance as a factor influencing the initial decrease in capillary resistance. We therefore thought it would be of interest to study pulmonary circulation in the closed chest prior to and after a single expansion of the lungs with nitrogen. The circulation was evaluated with indicator dilution technique and after pilot studies with indocyanine green only a technique was developed which makes use of two indicators simultaneously This paper is a report on the effect of lung expansion as evaluated with this double indicator technique.

Material and Methods

Thirteen lambs from nine ewes were used. Two of the lambs died early in the experiment because of hemopericardium caused by catheter perforation. In the remaining leven cases informative records were obtained.

(A) *Delivery of the lambs*

The lambs were delivered while the ewe lay on her right side. Under local anesthesia, the jugular vein of the ewe was dissected free and an initial dose of 0.4 to 0.6 grams of pentobarbital sodium was given intravenously Supplementary doses of 0.2 to 0.4 grams were given as needed to the ewe. A tracheotomy was done and a Harvard

Supported by grants from the United Cerebral Palsy Research and Educational Foundation (Dwight D. Eisenhower Fund) and the United States Public Health Service.

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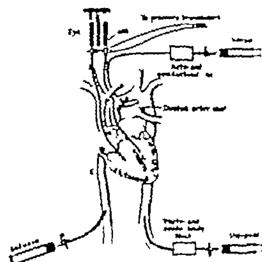


Fig. 1 Drawing showing how the distribution of right and left ventricular output were evaluated by recording the appearance in the right carotid artery and in the right femoral artery of two distinguishable indicators which had been injected simultaneously into the two ventricles.

respiration pump supplied 100% oxygen. Maternal arterial pH and pCO_2, determined by the micromethod of Astrup *et al.* [3], were maintained within values close to those in unanesthetized pregnant ewes.

The abdominal incision was para-median. To prevent prolapse of intestines, the edges of the abdominal incision were sutured to the uterus prior to the hysterotomy. The lamb was delivered on a heated table alongside the ewe tracheotomized, and particular care was taken not to disturb the cord circulation. One hundred mg of Heparin was administered into the fetal blood stream and the condition of the lamb was evaluated by pH, pCO_2, and pO_2 determination of the carotid artery blood with the Beckman Physiological Gas Analyzer —Model 160.[1]

(B) *Evaluation of the circulation*

The distribution of right and left ventricular output was evaluated by a rapid injection of indocyanine green and salt into right and left ventricles, respectively and recording the subsequent increase in concentration of those two indicators in the right carotid artery and the right femoral artery (Fig. 1)

The indocyanine green injected into the right ventricle was prepared by dissolving 50 mg dye with 15 ml of distilled water and then adding 10 ml of physiological saline. The dye solution thus prepared did not change the electrical conductivity of the blood. Approximately 0.5 ml, or 1 mg dye was given at a time. The amount was adjusted according to the size of the fetus and its cardiac output. The salt solution injected into the left ventricle was 5% sodium chloride given in the same volume as the dye solution. It did not cause any appreciable change in optical blood density.

Polyethylene catheters PE 50 (o.d. 1 mm) were introduced into the right jugular vein and the right carotid artery. They were connected to pressure transducers and the pressures were recorded on an eight channel Offner Dynograph. The catheters were advanced until the pressure tracings indicated that they were in the ventricles.

By turning the stopcocks marked C in Fig. 1 the catheters were disconnected from the pressure transducers and were made to communicate with the syringes containing the indicators (A). The plungers of the syringes moved in parallel and when they were rapidly pushed in, thereby emptying the syringes, an electrical circuit was closed which gave a mark on the recording paper. Since the catheters had previously been filled with the indicators, the amount of salt and dye ejected from the syringes was equal to the amounts introduced into the heart. Immediately after injection of the indicators, the catheters were again made to communicate with the pressure transducers. The reappearance of appropriate pressure amplitudes indicated that the tips of the catheters were still in the ventricles.

The blood withdrawn from the right carotid artery and the femoral artery passed in sequence the dye sensor, a densitometer

[1] Beckman Instruments, Incorporated, Fullerton, California.

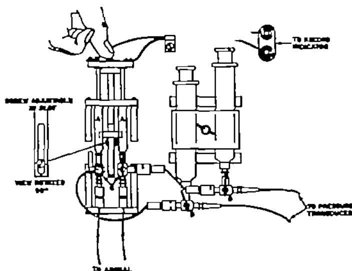


Fig. 2. Drawing showing device for simultaneous injection of two indicators. When the thumb pushed the plungers, this was indicated on the record. Large syringes marked B contained supplies of indicator solution.

and the salt sensor a conductivity cell. Because these sensing devices were in series, the appearance of the dye was recorded a quarter of a second prior to that of the salt.

Procedure

When the pressure tracings demonstrated that the catheters for injection of indicators were in the ventricles, withdrawal of blood at a rate of 8 ml/min was begun from the carotid artery and at the same rat from the femoral artery. In order not to change the fetal blood volume, blood was simultaneously infused into the right femoral vein at a rat of 16 ml/min. When the four tracings of indicator concentration were stable, the pressure transducers were disconnected by turning the stopcocks. The indicators were injected as fast as possible and the transducers were then reconnected. As soon as the required information was obtained, the blood from the arteries was returned and simultaneously withdrawal was started from the femoral vein. As a preparation for a new indicator dilution curve, the small indicator syringes were refilled from the supply syringes.

During a control period prior to lung expansion, an verage of four indicator dilution curves were obtained. The lungs were then expanded with 100-200 ml of nitrogen. A new indicator dilution curve was obtained 1–3 minutes after the trachea was opened, giving free outlet to any gas under pressure. In five cases the trachea was opened immediately after lung expansion, but in the remaining six cases the trachea was kept closed approximately 5 minutes, during which period 1 2 indicator dilution curves were btained. In two lambs the lungs were expanded with 100 ml of Dextran prior to expansion with an equal amount of nitrogen.

For calibration, whole blood was taken from the lamb at the end of an experiment. Standard dilutions of the indicators, according to the technique of Nicholson *et al.* [19], were drawn through the sensing devices at the same speed as during an experiment.

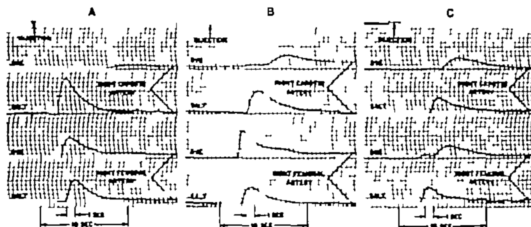


Fig. 3. Records obtained during the control period in three different lambs. Pulmonary blood flow was graded as 0 in A, as I in B, and as II in C.

Results

Generally the lambs appeared to be in good condition throughout the experiment and only in a few instances did the circulation through the umbilical cord seem to deteriorate. However not even these lambs tried to breathe during the experiments, but terminal clamping of the cord was invariably followed by gasps. The average arterial pH was 7.30 and the average values for pCO_2 and pO_2 were 44 and 23 mm Hg respectively.

(A) *Circulation during control period*

Fig. 3 exemplifies the three different types of indicator dilution curves ob-

TABLE I *Changes in pulmonary blood flow following lung expansion of the fetal lamb.*

Lamb No.	Weight in kg	Volume of nitrogen used for lung expansion (ml)	Pulmonary blood flow graded as 0, I, II or III (see text): During control period	During lung expansion	After lung expansion
1	3.0	100	0	—	0[a]
2	3.4	100	0	—	II[b]
3	3.5	100	I	—	II
4	3.6	100	0	0	0
5	3.9	100	0	0	III
6	4.0	100	I	I	II
7	4.3	100	0	—	II
8	4.3	200	0	0	I
9	4.6	100	II	II	I
10	4.8	200	I	0	II
11	4.9	100	I	—	III

[a] After a following expansion with 200 ml of nitrogen pulmonary blood flow increased from 0 to II.

[b] A previous lung expansion with 100 ml of Dextran did not affect pulmonary blood flow

served prior to any treatment. A minimal (A) a medium (B) and a large (C) pulmonary blood flow are demonstrated.

The tracings marked A were from a lamb weighing 3 0 kg (No. 1 Table 1). The minimal increase in dye concentration, observed in the right carotid artery appeared so late that it could be attributed to recirculation only As judged from this tracing, hardly any of the dye deposited in the right ventricle passed through the lungs. Instead, most of the right ventricular output went through the ductus arteriosus giving the high concentration of dye in the femoral artery The salt, injected into the left ventricle appeared in high concentration in the right carotid artery but was diluted by blood flowing into the aorta from the ductus, and thus its concentration was lowered in the femoral artery

The tracings marked B in Fig 3 were from a 3.5 kg lamb (No. 3) in which an ppreciable portion of the right ventricular output passed through the lungs. The increase in carotid artery dye concentration ppeared earlier and was greater than that due to recirculation only The dye curve from the femoral artery indicated that a minor portion of the right ventricular output passed through the lungs. Most of the dye took the short cut through the ductus to give the high and early appearing peak. However the delayed decrease in dye concentration after this peak could be mainly attributed to dye appearing after having passed through the lungs. In the right carotid artery there was a minor increase in salt concentration. This was interpreted as being due to a regurgitation of the dye from the right ventricle to the right atrium, and from there to the left side through the foramen ovale. It would seem conceivable that the unusually fast injection of indicators, in this case less than 0.2 sec provoked the regurgitation.

Tracings C in Fig 3 were from a 4.6 kg lamb (No. 9) and demonstrate the largest pulmonary blood flow observed in any animal during the control period. In the carotid artery the concentration of dye was almost as great as that of salt. The increase in dye concentration observed in the femoral artery was mainly due to the dye which had passed through the lungs, but the small plateau proceeding the main increase in concentration indicated that there was still some right to left shunt through the ductus. Because of the small amount of blood which flowed into the aorta from the ductus, the salt was approximately of equal concentration in the two arteries and not diluted in the femoral artery as in lambs A and B.

A quantitative evaluation of the circulation was originally planned but had to be abandoned since an evaluation of systematic errors revealed that the calibration had been performed without a satisfactory control of temperature Such a control is essential since electrical conductivity of blood is influenced not only by salt concentration but also by temperature. However for a detection of changes in the circulation, the method was well suited and the relative distribution of right ventricular output could be estimated from the two dye curves and was graded as 0 I II or III. When the increase in dye concentration in the carotid artery could be attributed to recirculation only the pulmonary blood flow was designated as grade 0 (Fig. 3A) When

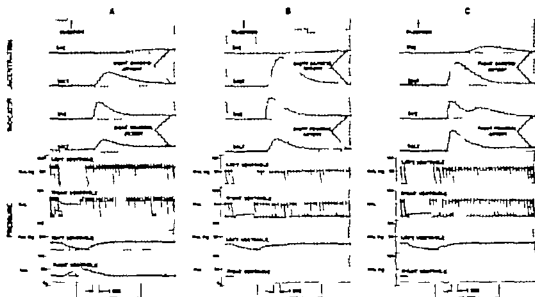


Fig. 4 Tracings from lamb No. 8 obtained during control period (A), during lung expansion (B), and after lung expansion (C) In tracings A and B, none of the right ventricular output passed through the lungs since no dye ppeared in right carotid artery In C the appearance of dy in the ight carotid artery and the double hump of dye in the right femoral artery demonstrat that a portion of the right ventricular output passed through the lungs.

there was a greater increase in dye con centration in the carotid artery than one due to recirculation only and when the femoral artery curve had two humps, the ulmonary blood flow was designated as de I if the first hump was the highest (Fig 3B) and as grade II if the second hump was the highest (Fig 3C) The pulmonary blood flow was designated as grade II when there was equal increase in dye concentration in the two arteries and no right to left shunt as judged from the absence of an appearance of dye in the femoral artery a quarter of a second prior to that of salt. This type of maximal pulmonary circulation was never seen during the control period.

Using this nomenclature the pulmonary blood flow during the control period was grade 0 in six lambs, grade I in four lambs, and grade II in one lamb (Table 1) The only spontaneous change in pulmonary blood flow observed during the control period was a decrease in flow from grade II to grade I observed in one lamb (No 10) In five lambs with clearly visible pulmonary blood flow the delay in dye appearance due to passage through the lungs and left atrium ranged from 1.3 to 3.3 sec. This time was corrected for the 0.5 sec delay in appearance of salt which was due to the fact that blood arrived later at the conductivity cell than at the densitometer

(B) *Circulation during lung expansion*

Expansion of the lungs without letting the nitrogen out again, invariably resulted in a decreased heart rate in the six lambs studied under these conditions. This

observation, together with that of a greater increase in salt concentration following an indicator injection, implied a decrease in cardiac output (Fig. 4B) The relative pulmonary blood flow decreased from grade I to grade 0 in one lamb but was unaffected in the other five.

(C) *Circulation after lung expansion*

When the trachea was opened to atmosphere again, the chest expansion was seen to decrease and a foamy material flowed out. In eight of the eleven lambs the indicator injection demonstrated that a greater proportion of the right ventricular output was now passing through the lungs. However in two lambs there was no change in distribution of right ventricular output and in one lamb (No 9 Table 1) the relative pulmonary blood flow decreased from grade II to grade I.

Thus, in the majority of cases (8 out of 11) the single expansion of the lungs with nitrogen, followed by deflation, resulted in an increase in pulmonary blood flow Fig 4 shows the typical sequence of events (lamb No. 8) The records marked A demonstrate the situation prior to lung expansion during the control period Injection of dye into the right ventricle and salt into the left ventricle revealed a minimal pulmonary blood flow In the right carotid artery there was hardly any increase in dye concentration, but a clear increase in salt concentration which demonstrated that the blood flowing through this vessel originated from the left ventricle only In the right femoral artery there was a conspicuous and simultaneous increase in dye as well as salt concentration, demonstrating that the right ventricular output took the short cut through the ductus arteriosus. Expanding the lungs (B) resulted in a decrease in cardiac output but no altered distribution of blood flowing from the right ventricle. When the gas had been given free outlet again (C) there was an obvious appearance of dye in the right carotid artery This observation, together with that of a second concentration increase of right femoral artery dye, demonstrated that there was now an appreciable portion of the right ventricular output which had passed through the lungs. However in the particular case illustrated in Fig 5 the first and rapid increase in concentration of right femoral dye demonstrated that there was still a considerable right to left shunt through the ductus arteriosus

As seen from Table 1 the most dramatic change in distribution of right ventricular output following lung expansion was observed in lamb No 5. The records from this particular lamb are shown in Fig 5. During the control period prior to lung expansion (Records A) dye appeared in high concentration in the femoral artery but not at all in the right carotid artery Thus, all the blood ejected from the right ventricle passed through the ductus arteriosus. In this case the two ventricles worked completely in parallel during the control period and continued to do so while the lungs were expanded with 100 ml of nitrogen. Shortly after this gas had been given free outlet again a new indicator dilution curve (B) demonstrated that the central circulation was completely altered. The dye appeared in equal concentration in the two arteries pproximately 1.25 sec later than the salt. This demonstrated that the entire right

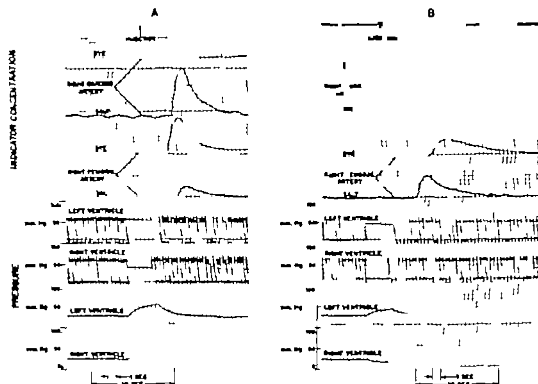


Fig. 5. Tracings showing the effect of lung expansion bserved in lamb No. 5. During control period (A) pulmonary blood flow was 0 but increased t III afte expansion with 100 ml of nitrogen (B).

ventricular output passed through the lungs. Prior to the lung expansion the were working in parallel but after the lungs had been expanded, the ventricles were working in series

In two lambs (No and No 7) the lungs were expanded with 100 ml of Dextran. This did not affect pulmonary blood flow although the following expansion with 100 ml of nitrogen in both instances caused an increase in pulmonary blood flow from 0 to II

Discussion

The purpose of these experiments was to evaluate fetal pulmonary blood flow under conditions which as far as possible, should be similar to those present in utero and furthermore to study the change of pulmonary blood flow as a result of lung expansion. Since only the mechanical effect of lung expansion was to be evaluated, the ideal gas for expanding the lungs would have been a mixture of oxygen nitrogen and carbon dioxide in the same proportions as present in the blood of the right ventricle However since the lungs were not ventilated but only expanded the use of nitrogen could hardly affect blood-gas tensions.

The central circulation of the fetal lamb should be evaluated with a method which in itself would cause a minimal interference with the circulation. Electromagnetic flowmeters have the advantage

of giving continuous information and when used in studies of fetal circulation have given valuable information [1 –, 6 16]. However for their application open chest surgery is needed and since several flowmeters would have been required, they would have occupied an appreciable part of the thoracic space which might have disturbed the circulation. Furthermore for reasons which will be given below it was considered particularly important not to open the thoracic cavity

Pulmonary vascular resistance is influenced by changes in arterial pO_2 and pCO_2 [6 8, 12 16] Acetylcholine or histamine causes vasodilatation [12] whereas noradrenaline or adrenaline has the opposite effect [7] Thus various chemical factors may influence pulmonary blood flow but the early observation made by Dawes *et al* [9] that purely mechanically lung expansion causes a decreased vascular resistance could be confirmed by Cassin *et al.* [6] by Lauer *et al.* [16], and is also confirmed with the experiments of this paper It is expansion of the lungs with a gas rather than expansion per se which causes an increase in pulmonary blood flow Thus, the operative factor must be the tension t the air liquid interface of the alveoli. This surface tension causes a pressure difference ΔP between the interior and the immediate outside of each expanded alveolus. The law of Laplace states that.

$\Delta P = 2\gamma/r$ where γ is surface tension and r is the radius of the alveolus.

When the lungs were forcibly expanded by a gas the pressure in the alveoli was increased whereby presumably some of the blood in the surrounding capillaries was squeezed out. The resulting reduction in the capillaries total cross sectional surface area should have increased their resistance. However when the gas in the airways was given free outlet again, the pressure in those alveoli remaining expanded returned to atmospheric. According to the law of Laplace, the pressure must be sub-atmospheric immediately outside those alveoli. Thus, after the lung expansion, pressure in the capillary network surrounding the alveoli should become less than in the peripheral vessels of the body In this way the pulmonary capillaries dilate, and their resistance to blood flow decreases. The mechanism is based on the concept that alveoli remain expanded after release of gas from the trachea It is conceivable that more alveoli remain expanded if the chest wall is intact. Therefore in these experiments intra-thoracic surgery was avoided. The principle of this mechanism is schematically presented in Fig. 0

This concept which has been presented previously by Lloyd & Wright [17], explains how surface tension may be the cause of the increase in pulmonary blood flow observed to follow ventilation of the lungs. Since all the other changes in the central circulation of the newborn may be secondary to the increased pulmonary blood flow surface tension may be the trigger to all these changes. The reduced resistance to passage of blood through the lungs lowers pressure on the right side of the heart When pressure on the right side becomes less than that on the left the valvular foramen ovale will close and the direction of blood flow through the ductus arteriosus will be reversed. With improving breathing, the blood delivered to the left atrium and thus flowing through the duc-

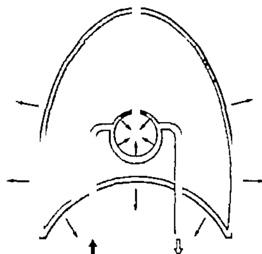


Fig. 6. Schematic presentation of concept of how surface tension may act to increase pulmonary blood flow. Since the air liquid inter face of the expanded alveolus tends to mov towards the center but is impeded from doing so by the diagraphm and chest wall, a negative pressure will be produced in the space surround ing the alveolus. Capillaries located i this space will dilate and their larger diameter will offer less resistance

tus will have an increasing oxygen tension and according to Dawes *et al* [4 10 11] and Assali *et al* [ ], this is the physiologic us for constriction of the ductus arteriosus in the lamb Recent studies by Moss *et al* [18] have demonstrated that also in the human newborn the ductus closes when it is traversed by blood with high oxygen tension but can be made to open up again by subjecting the individ ual to hypoxia. Similarly high oxygen tension in the blood of the umbilical ar teries causes these vessels to constrict [21], thereby cutting off placental circula tion. By reducing pulmonary vascular resistance surface tension in the aerated alveoli may thus be the trigger which closes the foramen ovale the ductus ar teriosus, and the umbilical arteries.

Fetal type of circulation requires a minimal flow through the lungs but a great flow down the aorta to the umbilical arteries. These requirements are fulfilled by the ventricles working in parallel. When the lungs become aerated and thus take over an important task from the pla centa the two ventricles should start working in series, instead. It would seem as if they might do so because of the sur face tension which appears in the lungs once they have been aerated. Already in utero pulmonary vascular resistance may be lowered by an increased pO_2, a dec reased pCO_2 or a release of acetylcholine [12] but the action of surface tension cannot be put at work until the newborn has taken its first breath and air liquid in terfaces have been created in the alveoli.

Summary

The change in pulmonary blood flow as a result of expansion of the lungs with 100–200 ml of nitrogen was studied in eleven fetal lambs. The fetal circulation was evaluated by simultaneous injection of 5% sodium chloride into the left ven tricle and indocyanine green into the right ventricle and by recording the con centration of these indicators in the right carotid artery and right femoral artery

During the control period, prior to lung expansion, most of the right ventricular output bypassed the lungs in fact, there was no detectable pulmonary blood flow in six of the lambs. As long as the lungs were forcibly maintained expanded there was no increase in pulmonary blood flow but when the expanding nitrogen was given free outlet again there was a change in the distribution of right ventricular

output in nine of the lambs. The portion passing through the lungs increased in eight lambs but decreased in one

In two lambs the lungs were expanded with 100 ml of Dextran. This did not affect pulmonary blood flow although the following expansion with 100 ml of nitrogen in both instances caused more of the right ventricular output to be distributed to the lungs.

It is concluded that surface tension in the newly formed air liquid interfaces of the expanded alveoli may decrease resistance to pulmonary blood flow

References

1. ASSALI, N S, SEHGAL, N and MARABLE, S.: Pulmonary and ductus arteriosus circulation in the fetal lamb before and after birth. *Amer J Physiol*, *202* 536, 1962.
2. ASSALI, N S, MORRIS, J A, SMITH, R W and MANSON A.: Studies on ductus arteriosus circulation. *Circ Res* *13*, 478, 1963.
3. ASTRUP P., JÖRGENSEN K., ANDERSEN O S. and ENGEL, K. The acidbase metabolism. A new approach. *Lancet*, *1* 1035, 1960.
4. BORN G.V R. DAWES, G S. MOTT J and RENNICK, B. R. The constriction of the ductus arteriosus caused by oxygen and by asphyxia in newborn lambs, *J Physiol*, *132* 304, 1956.
5. BROWN, E. S., JOHNSON R. P and CLEMENTS, J A.: Pulmonary surface tension. *J Appl Physiol*, *14*. 717 1959
6. CASSIN S., DAWES, G S. MOTT J C., ROSS, B. B. and STRANG L. B. The vascular resistance of the foetal and newly ventilated lung of the lamb. *J Physiol*, *171* 61 1964.
7. CASSIN S. DAWES, G S. and ROSS, B B.: Pulmonary blood flow and vascular resistance in immature foetal lambs. *J Physiol*, *171* 80 1964.
8. COOK, C. D DREYFUSS, P A. JACOBSON H. N LEVISON H. and STRANG, L. B Control of pulmonary blood flow in the foetal and newly born lamb. *J Physiol*, *169* 10, 1963.
9. DAWES, G S., MOTT J C., WIDDICOMBE, J G and WYATT D G. Changes in the lungs of the new-born lamb. *J Physiol*, *121* 141 1953.
10. DAWES, G S., MOTT, J C. and WIDDICOMBE, J G.: The fetal circulation in the lamb. *J Physiol*, *126* 563, 1954.
11. DAWES, G. S.: Changes in the circulation at birth. *Brit Med Bull*, *17* 148, 1961
12. DAWES, G S. and MOTT J C. The vascular tone of the foetal lung *J Physiol*, *164*. 465, 1962.
13. ENHÖRNING, G The surface tension of amniotic fluid. *Amer J Obstet Gynec*, *88* 519 1964.
14. ENHÖRNING, G., FUJIWARA, T and ADAMS, F H.: Surface-active material in fetal lung fluid concentrated by foaming. *Tohoku J Exp Med*, *84* 280, 1964.
15. ENHÖRNING G and ADAMS, F H. Surface properties of fetal lamb tracheal fluid. *Amer J Obstet Gynec*, *92*. 563, 1965.
16. LAUER, R. M., EVANS, J A., AOKI, H. and KITTLE, C. F Factors controlling pulmonary vascular resistance in fetal lambs. *J Pediat*, *67* 568, 1965.
17. LLOYD T C J and WRIGHT G W Pulmonary vascular resistance and vascular transmural gradient. *J Appl Physiol*, *15*. 241 1961
18. MOSS, A. J EMMANOUILIDES, G C. and ADAMS, F H.: Response of ductus arteriosus and pulmonary and systemic arterial pressure to changes in oxygen environment in newborn infants. *Pediatrics* *33*. 937 1964.
19. NICHOLSON III J W and WOOD, E. H. Estimation of cardiac output and Evans blue space in man, using an oximeter *J Lab Clin Med*, *38*. 588, 1951.
20. PATTLE, R. E. Properties, origin and function of the alveolar lining layer *Proc Roy Soc (Biol)*, *148*. 21 1958.
21. BOCK, W.: Untersuchungen uber den physiologischen Verschluss der Nabelschnurarterien. *Z Biol*, *81* 487 1925.

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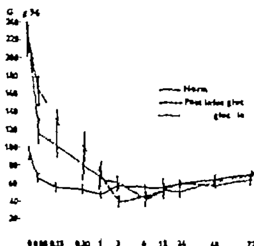


Fig. 1 Levels of glucose in the first 72 hrs. of life of the newborn after prenatal infusion of glucose and glucose with insulin or without infusions (norm.) into the mother

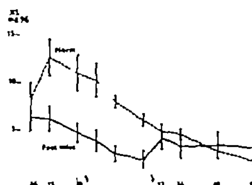


Fig. — Levels of excess lactate in capillary blood in the first 72 hrs. of lif in the group of normal newborns (norm.) and after infusions into the mother

blood at the moment of birth and umbilical vein blood levels (time 0) were quoted. The blood levels of lactate and pyruvate and the L/P ratio during the period of most rapid metabolic change showed some disturbances in their levels in the control group. The L/P ratio was higher than in both treated groups and disturbance of the lactate-pyruvate system reflected a lactate excess which increased in the controls.

Initial values of blood glucose in the infused groups were more than twice as high ($p < 0.01$) as in controls, with a continuous fall over the first postnatal hours. The lowest blood glucose values occurred 3 hrs. after glucose alone and 6 hrs. after glucose and insulin, after which periods levels were the same as in the control group (Fig 1)

In controls lactate excess increased in the first 6 hours with maximum at 15 min., i.e. there was more lactate than corresponded to a balanced state. This increased level of lactate excess was not observed in the infused groups. The lowest values of lactate excess were recorded after glucose-insulin infusions, the difference between this and the group without infusions being statistically significant ($p < 0.01$) for the entire period of 15 min. to 6 hrs. (Fig 2)

Oxygen consumption in all groups of

TABLE 2 *Mean values of oxygen consumption in ml O_2/kg/hrs Mean ± SE.*

Group	Time in hrs. 2	6	1	48
I. Glucose-insulin infusion	290 ± 29.50	291 ± 28.31	251 ± 10.79	282 ± 30.59
II. Glucose infusion	272 ± 20.60	273 ± 15.50	302 ± 60.49	320 ± 31.95
III. Control	315 ± 9.94	325 ± 10.83	355 ± 10.67	356 ± 9.99

newborns increased in a continuous fashion during first 48 hrs. (Table 2) with exception of the group after infusion of glucose with insulin at 1 hrs. There was a significant decrease ($p<0.01$)

Discussion

Dawes *et al.* [3] suggested that survival of asphyctic newborn infants depends largely upon cardiac and hepatic glycogen stores, and their availability as energy source. These depots of glycogen are depleted rapidly after birth. Simple glucose administration may not be effective, for under some conditions it may lead to increases in blood lactic acid and falls in pH [10] Cornblath [2] postulated that the brain of a newborn infant has a relatively large glucose consumption, and that in the presence of inadequate sources of glucose other tissues are forced into *employing non-carbohydrate energy* sources. Other compensatory mechanisms may come into play in this situation, such as reduction of oxygen requirements to the minimum.

Prenatal infusion of glucose with insulin, theoretically could supply the fetus with a readily available source of energy immediately before birth which could be utilized in the immediate postnatal period. Prenatal glucose infusion might also stabilize the relationship between blood lactate and pyruvate which shows deviation even following normal birth [13].

The Huckabee's formula for calculation of lactate excess takes account of all influences on the lactate-pyruvate system. Therefore it is possible, that a relatively small decrease of lactate in our group after maternal glucose infusion or a relatively small increase in blood pyruvate in our group after maternal glucose-insulin infusion was sufficient to reduce calculated ballast lactate excess.

One may assume that the rise of ballast lactate excess reflected a predominance of anaerobic metabolism at the period immediately after birth. Metabolic balance was not stabilised even by 3 hrs after birth when a transient fall in oxygen consumption was found in the control group, relative hypoglycemia persisted, body temperature reached its low point and the L/P ratio and XL were still not at normal levels. Under normal conditions it is only at the end of the third day that the L/P ratio reaches relatively low levels (9.97), blood glucose reaches normal values (69.14 mg/100 ml) and excess lactate virtually disappears (1.75 mg/100 ml)

Prenatal infusions of glucose and glucose with insulin probably influenced the metabolic state of the newborn infants by decreasing their need to mobilise fat from reserves after birth in order to cover energy needs. This view is supported by the fact that experimental administration of a source of energy has the same effect as a decrease in its expenditure. Such a decrease occurs, for example, when infant's body temperatures are maintained after birth by heating, as shown in a previous paper [9].

The so-called "acidotic constitution of the newborn as reflected by a rise in L/P ratio and ballast lactate excess after birth can thus be seen as a problem of energy balance Administration of easily consumed energy stores such as glucose with adequate amounts of insulin, might be able to influence withdrawals from

carbohydrate stores in the first hours after birth and facilitate the onset of respiratory control after birth.

Summary

10 women in labor received a drip infusion of 500 ml of 10% glucose with 16 units of crystalline insulin and further 10 received glucose infusion without insulin, about 30 min. before giving birth. In the newborn infants of these mothers was a continuous fall in the high initial blood glucose values in capillary blood, with significant decrease in ballast excess lactate without increase in oxygen consumption. It would appear that prenatal infusion of glucose with insulin decreased partially anaerobic metabolism without increasing the oxidative component. Such an effect might lower the tendency of the newborn to go into metabolic acidosis after birth, and assist the establishment of normal respiration.

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References

1. Barker, S. B. and Summerson, W. H.: The colorimetric determination of lactic acid in biological material. *Biol Chem*, 138: 535, 1941
2. Cornblath, M., Wybregt, A. S. and Baker, G. S.: Studies of carbohydrate metabolism in the newborn infant. *Pediatrics*, 32: 1007, 1963.
3. Dawes, G. S., Mott, J. C. and Shelley, H. J.: The importance of cardiac glycogen for the maintenance of life in foetal lambs and newborn animals during anoxia. *J Physiol*, 146: 516, 1959
4. Gitlin, G., Kumate, J. and Morales, C.: On the transport of insulin across the human placenta. *Pediatrics*, 35: 65, 1965.
5. Hášek, J., Kamaráš, M., Michalec, Č. and Slavík, K.: *Základy chemické lidské fyziologie*. St. zdrav. nakl., Praha 1957
6. Huckabee, W. E.: Relationship of pyruvate and lactate during anaerobic metabolism. *J Clin Invest*, 37: pp. 244–255, 264, 1958.
7. Joshkovich, J. B. and Kniazi, E.: Placental transfer of I[131]-insulin in the rhesus monkey. *Am J Physiol*, 200: 471, 1961
8. Nelson, N.: A photometric adaptation of Somogyi method for determination of glucose. *J Biol Chem*, 153: 375, 1944.
9. Přibylová, H. and Znamenáček, K.: The effect of body temperature on the level of carbohydrate metabolites and oxygen consumption in the newborn infant. *Pediatrics*, in press 1966.
10. Shelley, H. J.: Carbohydrate reserves in the newborn infant. *Brit Med J*, I: 273, 1964.
11. Šabata, V., Štembera, Z. K., Hodr, J. and Novák, M.: Die Wirkung von Glucoseinfusionen auf den Lipoid- und Kohlenhydratstoffwechsel der Gebärenden und der Frucht. *Z Geburtsh Gynaek*, 162: 33, 1964.
12. — Die Wirkung von Glucose- und Insulininfusionen auf den Lipoid- und Kohlenhydratstoffwechsel der Gebärenden und der Frucht. *Z Geburtsh Gynaek*, 164: 15, 1965.
13. Znamenáček, K. and Přibylová, H.: Some parameters of respiratory metabolism in the first 3 days after birth. *Acta Paediat Scand*, 53: 41, 1964.
14. Znamenáček, K. and Přibylová, H.: The significance of "blood excess lactate" in the neonatal period. *Acta Paediat Scand*, 53: 301, 1964.

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Measles Vaccination

VI Serological and Clinical Follow up Analysis 18 Months after a Booster Injection

by E. NORRBY R. LAGERCRANTZ, and S GARD

In previous papers in this series we described the results of immunization of children aged ½ to 2 years with three monthly doses of a formalin-inactivated alum-adsorbed vaccine [4] serological follow up 8 to 9 months after vaccination [5] and further follow up and administration of a booster injection 22 to 23 months after the primary immunization [6] The establishment of a long time persistence of antibodies after the primary immunization and of impressive booster responses both after injection of formalin-killed vaccine and purified hemagglutinin were shown. Varying degrees of resistance among vaccinees to natural challenge with regular measles were found during the time interval between primary immunization and booster inoculation. The antibody titers reached by the booster injections remained on high levels during an observation period of 8 months. The present paper describes results from clinical and serological follow-up studies during an additional period of 10 months.

Supported by grants from the Swedish Medical Research Council (Project No. B66-234).

Material and Methods

Study population. The group of children given their primary immunization with three monthly doses at the age of ½ to 2 years [4] and revaccinated 22 to 23 months later [6], were inquired about exposures to measles during the time interval between 8 and 18 months after the booster On these two occasions samples of whole blood were collected from the finger tip and mixed with phosphate buffered, pH 7.2, physiological saline containing heparin. After removal of erythrocytes by centrifugation and inactivation at 56°C for 30 minutes the sera were used in serological analyses.

Serological analyses. The techniques for hemagglutination-inhibition (HI) and complement-fixation (CF) test previously described in detail [4, 5] were used. To permit accurate comparison sera from the 8 and 18 months post-booster bleedings were tested simultaneously Reference sera were included in the tests to allow comparison with earlier data. All titers given refer to final dilutions after addition of antigen.

Results

Clinical and serological analyses of effects of known or presumed exposure during 8 and 18 months after a booster in

TABLE 1 *The clinical and serological reactions in 11 children exposed to clearcut cases of measles during a time period of 8 to 18 months after a booster injection. In addition No 46 was included in the table since this child exhibited a marked rise in antibody titers although no known exposure to measles had occurred*

Child No.	Type of vaccine given as booster	Time for exposure months after booster	Clinical reaction	8 months post booster serum titers HI	8 months post booster serum titers CF	18 months post booster serum titers HI	18 months post booster serum titers CF
3	FK	14	0	160	< 20	20,480	640
6		15	0	640	20	10,240	320
19		15	0	160	< 20	10,240	640
22		13	See separate case history	640	20	128,000	1280
39		14	0	1280	40	640	20
46		?	—	1280	40	10,240	320
62		14	0	160	20	80	20
7	TE	16	0	20 480	1280	20,480	640
20		13	0	20,480	640	10,240	320
21		14	0	20,480	640	10,240	320
34		12	0	10,240	320	640	320
48		13	0	10,240	640	5120	320

fection Eleven out of 21 children available for follow up at 18 months after injection of a booster dose had been clearly exposed to measles during the last ten months. The source of exposure was close contact either with siblings or playmates, who had contracted typical measles. In Table 1 data are summarized on these 11 vaccinees plus one more No 46 The parents of this latter child did not know of any contact with measles or occurrence of symptoms indicating measles, but in spite of this a marked increase in both HI and CF antibody titers was recorded. None of the children displayed any symptoms clearly attributable to the exposure except one child, No 22 The history of this case was as follows:

Child No. 22 had received formalin-killed (FK) alum-containing vaccine both for primary immunization and as a fourth injection. Thirteen months after the latter a sister of this girl contracted regular measles. Nine days after this exposure the vaccinee came down with some coughing and fever ranging between 38 and 39 C. After three more days she had recovered somewhat but two days later the symptoms became aggravated. The fever rose again and reached 40 C and the severity of the symptoms from the respiratory tract increased markedly A visiting physician diagnosed a bronchopneumonia and prescribed penicillin. No X-ray examination of the chest was made The child finally recovered after three more days. No clear signs of measles, such as Koplik spots and rash, were found during the illness. *However as can be seen from Table 1 there was an impressive increase in antibody titers*, which strongly suggests that the pneumonia directly or indirectly was associated with a measles infection.

Although none of the remaining children exposed to measles displayed any symptoms, serologic responses varied. Among children who had received four injections

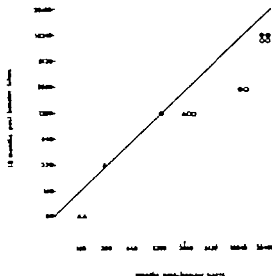


Fig. 1 Correlation between HI serum titers 8 and 18 months after booster injection of FK (Δ) or TE (O) vaccine. A filled symbol indicates that the vaccinee has been exposed to measles

of FK vaccine four out of six exhibited definite antibody rises, indicating the occurrence of symptomless infections in spite of the presence of rather high pre-exposure titers. No clear correlation between the degree of protection against invasion and possible spreading of virus and pre-exposure antibody titer levels could be distinguished. However the absence of detectable changes in serum titers after exposure to measles of children, who had received Tween-ether (TE) vaccine and whose pre-exposure antibody titers were exceptionally high, suggests the presence of a certain relationship

Changes in antibody titers during a period between 8 and 18 months after a booster injection. The changes in antibody titers in some of the vaccinees, who had been exposed to measles were already shown in Table 1 The concentrations of HI and CF antibodies in sera from all 21 children available for both 8 and 18 months follow up analyses are illustrated in Fig 1 and _, respectively With the exception of the paired sera from the above-mentioned child No. 46 there were no or only slight reductions with time in antibody titers of unexposed children. This also holds true for paired sera from children, among whom no reaction to exposure was demonstrable. The general trend was a one dilution step reduction in titer during the time period of 10 months. A comparison of mean titer values of 8 months postbooster sera previously published [6] and those obtained in the present retitrations performed for the sake of comparison demonstrated a high degree of reproducibility in the tests.

Correlation between HI and CF antibodies 18 months after a booster injection Fig. 3 shows the correlation of HI and CF antibodies in sera collected 18 months after the booster injection. Although there was for various reasons a considerable

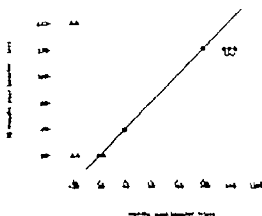


Fig. 2. Correlation between CF serum titers 8 and 18 months after booster injection. Symbols in Fig. 1

variation in absolute titers of individual sera a certain degree of correlation between values obtained in the two test was apparent. The ratio between HI and CF antibody titers appeared similar both in exposed and unexposed children. It varied from about 5 fold in children given a booster of TE vaccine and down to about 10 fold in children who instead received FK vaccine and who had not been exposed to measles.

Discussion

Immunization with four doses of killed measles vaccine appeared to have conferred a solid protection against clinical reactions in almost all cases exposed to regular measles in the present study. The only exception was the girl who contracted bronchopneumonia. It is interesting that once previously in these studies a similar case was encountered [6] and that also Rauh & Schmidt [8] recently described three cases of bronchopneumonia which occurred in children thoroughly immunized with killed vaccine. The diagnoses of the latter three cases were confirmed by X ray examination. The etiology of this adverse reaction to measles exposure of some children with circulating antibodies can at present only be a matter of speculation. One possible explanation could be that it is related to a localized reaction between antigen and antibody as proposed for cases of variola-immune individuals contracting a "virus pneumonia syndrome" [1]. Such an explanation for cases of measles pneumonia has been proposed in other connections [2].

Evidently varying degrees of resistance against natural measles challenge can be achieved by immunization with a killed vaccine only. Comparatively high HI antibody titers are required to give clinical protection and even higher titers to prevent not only appearance of symptoms, but also a detectable multiplication of virus. As was discussed previously [7] it appears as if much lower titers of circulating HI antibodies derived from administration of gammaglobulin are required to give a corresponding degree of protection. This was

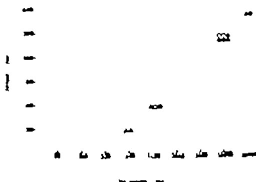


Fig. 3. Correlation between HI and CF serum titers in blood samples collected 18 months after a booster injection. Symbols as in Fig. 1

also concluded from a parallel study undertaken by Karzon *et al.* [3] The reason for this presumably qualitatively varying protective effect of HI antibodies of different origin is not known at present. This problem should be submitted to a careful analysis.

The experience that after immunization with only killed vaccine comparatively high antibody titers have to be obtained in order to provide vaccinees with a protection against virus multiplication does not preclude the use of such a vaccination regime. In a number of the children included in the present trial such a solid state of immunity has apparently been reached. The duration of this immunity is at present not known, but the stabilization of antibody titers on high levels over a period of 16 months after the booster injection is very encouraging. For natural reasons the outcome of an immunization with a killed vaccine only is completely dependent upon the schedule of immunization used. What concerns measles vaccination the relative importance of several parameters, such as (a) number and spacing in time of injections, (b) dose of antigen, (c) presence or absence of adjuvant and (d) combination with other antigens, remains to be carefully analysed in one and the same comprehensive field study.

It has been described [9] that after natural measles the decrease in serum titers with time is larger for CF antibodies than neutralizing or HI antibodies. It is interesting that a similar phenomenon does not seem to occur after immunization with a killed vaccine. Thus the ratio of HI to CF antibody titers remained approximately constant in sera collected after primary immunization [4], eight [5] and as found here 18 months after a booster injection. The significance and implications of this finding are at present not known.

Summary

Twenty-one children given three monthly doses of a formalin killed vaccine at the age of ½ to _ years and a booster of the same product or purified hemagglutinin 22 months later were submitted to a serological and clinical follow up 18 months after the booster.

Eleven children had been exposed to measles. Only one of these displayed symptoms. This case showed an atypical behaviour and was associated with a bronchopneumonia of short duration. Increase in antibody titers indicated symptom-less infections in four cases with and one without known exposure.

During the time 8 to 18 months after the booster the average decline in HI and CF antibody titers was about two-fold.

Acknowledgements

The excellent technical assistance of Miss Britt Åkestad, Miss Birgitta Nordn and Mrs Birgitta Lindström is gratefully acknowledged.

References

1. Dixon, C. A.: *Smallpox.* J & A Churchill Ltd London, p. 149 1962.
2. Kempe, C. H. and Fulginiti, V. A. The pathogenesis of measles virus infection. *Arch ges Virusforsch, 16* 103, 1965.
3. Karzon, D T Rush, D and Winkelstein, W Jr Immunization with inactivated measles virus vaccine: effect of booster dose and response to natural challenge. *Pediatrics, 36* 40, 1965.

4. NORRBY E., LAGERCRANTZ, R., GARD S. and CARLSTRÖM, G.: Measles vaccination I. Serologic responses to an inactivated vaccine. *Arch ges Virusforsch, 13:* 848, 1963.
5. NORRBY E., CARLSTRÖM, G LAGERCRANTZ, R. and GARD S.: Measles vaccination II. Antibody titers 8 to 9 months after vaccination with an inactivated accine *Acta Paediat Scand, 53* 533, 1964.
6. NORRBY E., LAGERCRANTZ, R. and GARD, S.: Measles vaccination IV Responses t tw different types of preparations given as a fourth dose of vaccine. *Brit Med J I* 813, 1965.
7 NORRBY E., LAGERCRANTZ, R. and GARD, S.: Measles vaccination V The booster effect of purified hemagglutinin in children previously immunized with this product or formalin killed vaccine. *Acta Paediat Scand, 55* 73, 1966.
8. RAUH, L. W and SCHMIDT R.: Measles immunization with killed virus vaccine. *Amer J Dis Child, 109* 232, 1965.
9 STOKES, J., JUN., REILLY C M., BUYNAK, E. B and HILLEMAN, M. R.: Immunologic studies of measles. *Amer J Hyg 74* 293, 1961.

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Growth in Congenital Adrenal Hyperplasia

by C. G BERGSTRAND

It is well known that linear growth in the adrenogenital syndrome associated with congenital hyperplasia of the adrenal cortex is markedly accelerated. Since the rate of skeletal maturation exceeds the rate of increase in height, the period of growth is abbreviated and the ultimate height of the individual is generally below average. It could be expected that suppression of the abnormal androgen production by treatment with cortisone or its analogues, would normalize the rate of linear growth and prevent the undue acceleration of skeletal maturation. For an evaluation of the final results of the treatment with cortisone with regard to growth and development a long period of observation is necessary As this treatment was first introduced in 1950 the number of patients treated since infancy or early childhood, and followed for a sufficient number of years, is necessarily limited. Nevertheless it seems possible to draw certain conclusions to day regarding the influence of cortisone treatment on linear growth in congenital adrenal hyperplasia.

Materials

The material consists of 28 patients, 14 girls (female pseudohermaphrodites) and 14 boys ranging in age at the time of the study from 8 to 26 years. In addition data for an untreated boy who died at an age of 4 years are presented to illustrate the growth rate during the first years of life in untreated cases. Of these 29 patients, 9 (including the boy who died at 4 years) showed a more or less severe salt-losing syndrome. As far as could be established, none had the hypertensive form of the congenital adrenogenital syndrome. Two of the female pseudohermaphrodites (patients L. J and B.S.) had completely virilized external genitalia with a well-developed penis and an empty but otherwise typical scrotum. Both had been raised as boys and had not received any treatment. In both cases a laparotomy had shown normal female internal genitalia. The majority of the patients had been examined by the author and some had been under his personal supervision for many years. By courtesy of other childrens hospital it was possible to obtain information concerning the rest of the patients. Data was also collected from school nurses and parents. It was not possible in all cases to make a complete reconstruction of the growth curve and in a few cases only information regarding final height was obtainable. The degree of skeletal maturation had not been studied sufficiently in all cases, and in a few patients there was some doubt that the instructions of treatment had been strictly followed. In spite of these shortcomings, however the material seems sufficient to illustrat the different types of growth and development en-

[illegible]LE 1 *[illegible]dequately treated boys*

Patient	Date of birth	Age at beginning of treatment	Bone age at beginning of treatment yr	Type of treatment	Age when linear growth ceased yr	Age at epiphyseal closure yr	Actual age yr	Actual height in cm	Final height in cm	Saltloser	Heredity
D K A.	4 65	3 w	normal	[illegible] he	—	—	10	136	—	+	Mother 161 cm Father 176 cm
R I	9 4 48	4½ y	13 14	[illegible]	13	13	17	163	163	—	Mother 157 cm Father 165 cm
T L.	17 [illegible] 51	3 yr	[illegible] anced	[illegible] he	—	—	14	131		[illegible]	Mother 148 cm Grandfather 157 cm
A.H.	6.9.48	4 yr	11 [illegible]			—	17	167	167		Mother 155 cm Father 172 cm
T.H.	16.[illegible].53	[illegible] yr	4				13	157			Mother 155 cm Father 17[illegible] cm
I U	12.[illegible].51	[illegible] yr	10			—	14	156			Mother 160 cm Father 165 cm

Abbreviations: [illegible] cortisone; he [illegible] cortisol; pr prednisolone; d dexamethasone; t triamcinolone

Patients A.H. and T.H. are brothers

TABLE [illegible] *[illegible]dequately treated girls (female pseudohermaphrodites)*

Patient	Date of birth	Age at beginning of treatment	Bone age at beginning of treatment	Type of treatment	Age when linear growth ceased y	Age at epiphyseal closure yr	Actual age y	Actual height in cm	Final height in cm	Saltloser	Heredity
U B.F	1 7.56	1	normal		—	—	9	138	—	+	Mother 160 cm Father 183 cm
B.N	6.8.55	[illegible] m	normal	[illegible] pr	—	—	10	131	—	+	Mother 161 cm Father 172 cm
E.M	10.4.56	2 m	normal		—	—	9	120	—	+	Mother 173 cm Father 178 cm

TABLE 3 *Boys untreated or treated after the age of six*

Patient	Date of birth	Age at beginning of treatment yr	Bone age at beginning of treatment yr	Type of treatment	Age when linear growth ceased yr	Age at epiphyseal closure yr	Actual age yr	Actual height in cm	Final height in cm	Saltloser	*Heredity*
B.K.	18.11.53	6	15–16	+dx	10	10–11	11	156	156	—	Mother 160 cm Father 164 cm
A.L.	16.10.51	7	15?	hs	12	13–14	14	16_	163	—	Mother 164 cm *Father 164 cm*
S.L.B.	27.12.53	8	?		10	?	12	162	162	+	Unknown
L.T.	14.9.51	10	16–17		13	13	13	165	165	—	Mother 157 cm *Father 181 cm*
J.A.	7.6.54	6	16?	+ dx	10	beginning at 10	11	163	163	—	Mother 160 cm Father 170 cm
H.O.Ö.	31.6.49	8	14–15	pr + dx	—	—	15	165	—	—	*Mother 153 cm* F ther 172 cm
P.A.R.	24.2.39	—	—	X treatment		10½	26	163	163	—	Unknown
L.E.H.	17.4.42	—	—		?	—	22	150	150	—	Mother 163 cm Father 181 cm
B.O.H.	19.3.49	—	—		—	—	—	—	—	+	—

Abbreviations as in Table 1

Twinbrother of patient K.T. table 4
Brother of patient B.B. table 4.
Dead at 4 yr

TABLE 4 *Girls (female pseudohermaphrodites) untreated or treated after the age of five*

Patient	Date of birth	Age at beginning of treatment yr	Bone age at beginning of treatment yr	Type of treatment	Age when linear growth ceased yr	Age at epiphyseal closure yr	Actual age yr	Actual height in cm	Final height in cm	Saltloser	Heredity
H.M.A.	22.5.47	7	?	pr	11	13	18	153	153	—	Unknown
G.A.	12.12.42	12	?		10–11	?	22	152	153	—	Mother 161 cm Father 178 cm
B.A.	6.1.56	6½	13	+dx +fr	9–11	—	10	134	—	—	Mother 161 cm Father 172 cm
I.B.	2.2.49	8	?	c[a]	11	11	16	158	158	—	Unknown
A.H.	6.12.46	7	?		11–12	10	18	143	148	—	Mother 16[illegible] cm Father 172 cm
B.P.	9.1.45	14–15	?	c[a]	<14	?	20	158	158	—	Unknown
K.P.	4.7.51	11	13		11–12	11	14	151	151	—	Mother 154 cm Father 179 cm
M.L.S.	6.1.50	7	?	pr	12	11–12	16	146	146	—	Unknown
L.T.[b]	14.9.51	10	13		12	11	12	149	149	—	Mother 150 cm Father 169 cm
D.S.	9.10.47	—	—	treatment	?	?	17	153	153	—	Mother 143 cm Father 184 cm
L.J.	4.11.44	—	—		13	?	20	157	157	+?	Mother 163 cm Father 174 cm

Abbreviations as in Table 1

[a] Inadequate treatment.
[b] Twin sister of L.T. table 2.
'completely' virilized, raised as males.

TABLE 5. *Birth weight and length in congenital adrenal hyperplasia compared with normal Swedish newborns.*

		Birth weight in g			Length at birth in cm		
		n	Mean	S.D.	n	Mean	S.D.
Adrenogenital syndrome	m	23	3796	616	21	51.7	2.0
	f	26	3538	610	22	50.8	2.2
Normal	m	32,066	3596	542	32,068	51.4	2.4
	f	26,893	3406	503	26,893	50.3	2.3

countered in congenital adrenal hyperplasia. Relevant data of the patients are given in Tables 1–4.

The 23 patients who had been followed for a length of time permitting an evaluation of their linear growth were divided in two groups. In the first group ("adequately treated") were included the patients who had been treated from a relatively early age (boys from 3 weeks to 4½ years, Table 1; girls from 1 to 2 months, Table 2). The daily 17-ketosteroid excretion had in these patients generally been maintained at an acceptable level i.e. according to Bongiovanni [3] between birth and five years of age 2 to 3 mg, between six and ten years 4 to 8 mg and after this age 10 to 18 mg. As seen from the tables most patients received cortisone or cortisol. In a minority cortisone was exchanged for prednisolone or dexamethasone.

The second group ("inadequately treated") consisted of patients who either had received no treatment at all or in whom treatment was instituted after the age of five to six years (Table 3 and 4). In four of these patients (S.E.S., B.M.A., I.B. and B.P.) it could be suspected or was known that therapy had been neglected during certain periods. Most of the patients in this group had been treated with cortisone. The steroid doses varied individually and with age. In some patients rather high doses were employed, at least for a short time, but with very few exceptions no signs of overdosage were observed. For maintenance treatment the steroids were given orally. The highest daily dose of cortisone employed was 100 mg and the lowest 15 mg. The corresponding dose range for prednisolone and dexamethasone was 15 to 4 mg and 1.5 to 0.5 mg respectively.

When available, X ray films of the patients were collected and reevaluated with regard to bone age. Skeletal age was determined according to Elgenmark [4] for the youngest age group and according to Schinz *et al.* [7] and to Tanner & Whitehouse [8] for the older children. The latter method gave a slightly lower skeletal age than that of Schinz *et al.*

Information about birth weight, length at birth and gestational age was available for a number of patients with congenital adrenal hyperplasia. In most cases these data were collected from hospital records; only a few patients had been born at home. The average figures for birth weight and length were compared with the corresponding normal Swedish figures g en by Engström & Falconer [5].

Results

The average birth weight and length for the patients with congenital adrenal hyperplasia and the corresponding normal values for newborn Swedish infants are presented in Table 5. There is no difference between these two groups with regard to length t birth. The average birth weight of the infants with congenital adrenal hyperplasia is somewhat higher but the differ

This part of the investigation was made in collaboration with Kristina Ekengren M.D. whose help is gratefully acknowledged.

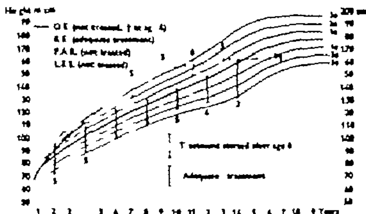


Fig. 1. Linear growth of boys with adrenogenital syndrome. Vertical lines represent distribution of height at given age and figures above and below indicate number of individuals.

ence is not significant. Gestational age was in most cases normal and a lack of agreement between birth weight and duration of pregnancy could not be established.

Linear growth of the patients is presented in Fig. 1 and 2. The available data are plotted against the growth curve of normal Swedish boys and girls. The vertical lines in the diagrams represent the distribution of height of the patients at a given age. The number of individuals, "adequately" or "inadequately" treated, is denoted by the figures below and above these lines. As seen in the diagrams there is a considerable difference between the growth curves of the two groups. This seems to hold true both for the boys and the pseudohermaphrodites. The patients whose treatment had been instituted after the age of 5 to 6 years show initially a rapid linear growth which decreases in spite of the treatment. Of the boys in this group all had reached their final height at the time of the follow-up study with one possible exception (patient H. O. Ö.). Growth had ceased between ages 10 and 13

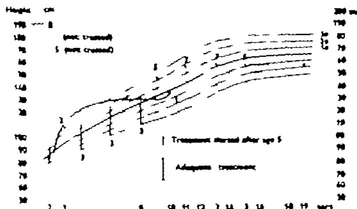


Fig. 2. Linear growth of girls with adrenogenital syndrome. Symbols as in Fig. 1.

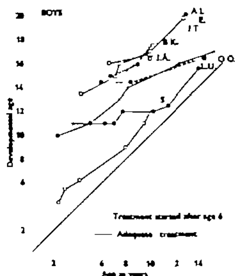


Fig. 3. Skeletal maturation of boys with adrenogenital syndrome.

(Table 3). Epiphyseal closure could be demonstrated by roentgenological examination except in patient H.O Ö whose distal femoral and proximal tibial epiphyseal cartilages were not yet ossified.

The inadequately" treated girls ceased growing between ages 10 and 1° (Table 4) Patient B.A. the youngest girl in this group showed an exceptional growth curve for which no definite explanation is at present available.

The growth curve of the "adequately" treated patients was generally normal. There was however one exception. The boy R.E. (Fig. 1) who had been treated since the age of 4½ year showed a linear growth corresponding to the curves of the inadequately" treated patients. He was also the only one in this group of six who had an early epiphyseal fusion.

In the inadequately" treated group, 7 boys and 10 girls had reached their final height which, for the boys ranged between 160 and 165 cm and for the girls between 145 and 158 cm.

Skeletal development in the patients who had been subjected to regular roentgenological examinations is presented in Fig 3 and 4. As seen from the graphs bone age was advanced in all the boys. This was less pronounced in the adequately treated group with one exception for patient R.E. Two of the girls who had been treated from a very early age had, up to the time of the study a normal skeletal development. The third patient in this group (U B.E.) had, at 5 years a bone age corresponding to 1* years but as seen from the graph further development was at least temporarily arrested. It should be noted that the differences in skeletal maturation at the developmental age of 18 to °0 years as presented in the graphs are small and of little significance.

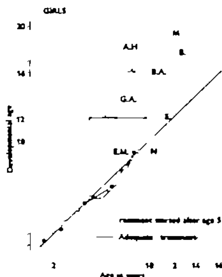


Fig. 4. Skeletal maturation of girls with adrenogenital syndrome.

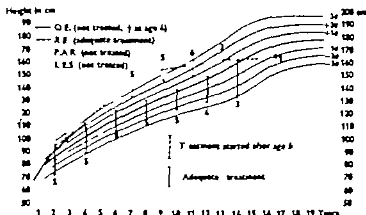


Fig 1 Linear growth of boy with adrenogenital syndrome Vertical lines represent distribution of height t given ge and figures above and below indicate number of individuals.

ence is not significant Gestational age was in most cases normal and a lack of agreement between birth weight and duration of pregnancy could not be established.

Linear growth of the patients is presented in Fig 1 and .. The available data are plotted against the growth curve of normal Swedish boys and girls. The vertical lines in the diagrams represent the distribution of height of the patients at a given age. The number of individuals, "adequately" or inadequately" treated, denoted by the figures below and above these lines. As seen in the diagrams there is a considerable difference between the growth curves of the two groups. This seems to hold true both for the boys and the pseudohermaphrodites. The patients whose treatment had been instituted after the age of 5 to 6 years show initially a rapid linear growth which decreases in spite of the treatment. Of the boys in this group, all had reached their final height at the time of the follow-up study with one possible exception (patient H.O Ö) Growth had ceased between ages 10 and 13

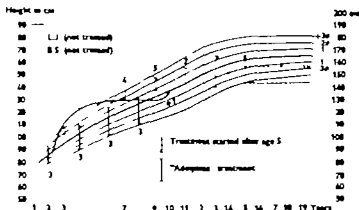


Fig. 2. Linear growth of girls with adrenogenital syndrome Symbols as in Fig. 1.

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The Behaviour of Foetal Haemoglobin in the enhanced Heinz Body Formation in Red Cells of Newborn Infants

by LAMIA ULUKUTLU ENNO KLEIHAUER and KLAUS BETKE

Recent observations have shown that newborn infants, especially prematures, are more liable to form Heinz bodies within their erythrocytes than are adults [4, 11]. This pronounced susceptibility can also readily be demonstrated in vitro by incubating blood with acetylphenylhydrazine. Zinkham [12] established that in the first day of life hypoglycaemia causes an instability of reduced glutathione by impairing the activity of glucose-6-phosphate dehydrogenase However this does not give an adequate explanation for the increased Heinz body formation in the newborn's erythrocytes since this phenomenon is not only present on the first day of lif but for several weeks thereafter Furthermore Tyoa [10] demonstrated in experimental studies that the enhanced Heinz body formation in red cells from newborn infants is not abolished by maintaining a high level of reduced glutathione by adding glucose

Red cells of newborns contain mainly foetal haemoglobin (HbF) and a much smaller amount of adult haemoglobin (HbA) HbF was found to be more susceptible to oxidization to methaemoglobin than is HbA [2 3] One wonders whether oxidative denaturation might also be enhanced in the case of HbF This question has been studied in the investigations reported below

Material and Methods

Blood was collected from the cord immediately after ligation. Blood of adults and f two newborn infants was obtained by venipuncture. Citrate was added as anti coagulant Determinations were performed within 4 hours of sampling in all cases.

1 *Denaturation of haemolysates.* Red cells washed three times with isotonic saline were haemolysed by adding 1½ vol. of water and ½ vol. of carbontetrachloride and shaking After centrifugation for 15 min t 6000 rpm the clear supernatant was diluted to a concentration of 10 g/100 ml by adding water

1 ml of haemolysate and 0.5 ml of 1 % solution of acetylphenylhydrazine in isotonic NaCl phosphate buffer of 7 4 were mixed in several test tubes. The samples were incubated t 37° in a water bath. After 10 min for temperature balance and 2, 4 and 8 hours later one sample was taken out. After precipitation of denatured haemoglobin by adding 1 ml of a saturated solution of ammoniumsulfat the concentration of haemoglobin was determined using the cyanmethaemoglobin method and a Zeiss spectrophotometer at a w ve-length of 540 nm. All runs were performed in dupli-

Fellow of the Alexander von Humboldt Stiftung.

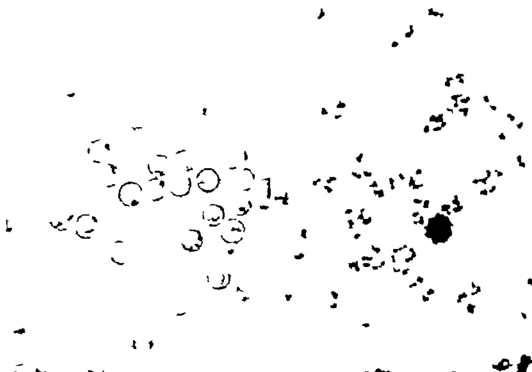


Fig. 1. Heinz body formation within erythrocytes from adults (left) and from newborns (right) after incubation of citrated whole blood with 0.33 % acetylphenylhydrazine at pH 7.4 and 37° for 3 hours.

cate and adult and newborn samples were run in parallel.

— *Heinz bodies in HbF-cells and HbA-cells.* In these experiments Heinz bodies were induced in whole blood *in vitro.* After preparing smears the individual red cells were examined for the presence of Heinz bodies and whether they contained HbF or HbA, using the acid elution method of Betke & Kleihauer [1].

1 ml of freshly drawn citrated blood was mixed with 0.5 ml of the above 1 % solution of acetylphenylhydrazine and incubated for 3 hours at 37°. One drop of the sample was mixed with one drop of a 1 % nile-bluesulfate solution in isotonic saline and kept in a moist chamber for 40 min. After preparing smears multiple spots were microphotographed using oil immersion ("photo 1"). The smears were cleaned with toluene fixed with 80 % ethanol, and eluted in a citric acid phosphate buffer of pH 3.3 at 37° in order to remove HbA from the red cells. After elution the same spots of the smears were microphotographed a second time ("photo 2"). Photo 1 was used to count Heinz bodies formed within the cells, photo 2 of the same spot served for differentiating the cells whether they contained HbF, HbA or both pigments.

Results

1. *Denaturation of haemoglobin by acetylphenylhydrazine.* The results of 10 experiments are summarized in Table 1. As can be seen denaturation proceeded slowly in both haemolysates. Within 8 hours 20.84 % (S.D. $\pm$ 4.78) of the blood pigment was denatured in the cord hae-

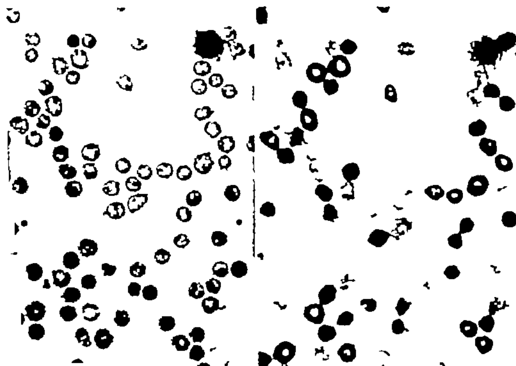


Fig. 2. *Left:* Erythrocytes of a 14-day-old newborn infant with Heinz bodies after incubation with acetylphenylhydrazine. *Right:* Same spot of the smear after application of the acid elution technic in order to differentiat HbF and HbA-cells.

molysates and 21.56 % (S.D. ±3 74) in the adult haemolysate The difference is statistically significant ($p < 0.02$).

2. *Heinz body formation in HbF-cells and HbA-cells.* Fig. 1 gives an impression of the results The smears of adult blood showed very small Heinz bodies within the cells, mostly a single one only Heinz bodies in the smears of two newborn in fants aged 11 and 14 days were coarse and in much greater number On first inspection there seemed to be no difference between HbF-cells and HbA-cells in the newborn (Fig 2). However in a total of 12.806 HbF-cells (noneluted in photo 2) an average of 5.6 (range 1–17) Heinz bodies was counted, whereas in a total of 2054 HbA-cells (eluted in photo) an average of 4.8 (range 1–11) was found Partly eluted cells (HbF + HbA) showed in the mean 5.5 Heinz bodies.

TABLE 1 *Denaturation of haemoglobin in haemolysates of cord erythrocytes and adult erythrocytes by incubation with acetyl phenylhydrazine at 37°C*

	Percent haemoglobin denatured after		
	2 hours	4 hours	8 hours
Cord			
mean	10.61	17.08	26.84
range	(3.9–13.6)	(10.7–22.1)	(19.2–33 4)
Adult			
mean	9.21	14. 8	21.56
range	(6.1 12.6)	(9.8–18.8)	(15 3–29.0)

Discussion

Heinz bodies seem to be made up mainly of denatured haemoglobin [6 7] however stroma constituents may be involved [8 9]. In our experiments the denaturation of newborn haemoglobin by acetylphenylhydrazine proceeded somewhat more rapidly than that of adult haemoglobin, with a relative rate of approximately 5 4 Considering the fact that the denaturing action of acetylphenylhydrazine is brought about by an oxidative process this small difference is surprising Oxidation to methaemoglobin by oxidizing agents such as potassium ferricyanide or sodium nitrite proceeds at double the rate in HbF compared with HbA [2, 3] Methaemoglobinization of the blood pigment within the red cells by acetylphenylhydrazine takes place at a relative rate of 8 2 in erythrocytes of newborns and of adults as shown by Gröschner [5].

On the other hand Heinz body forma- m was much more intense within red l of newborns than in adult cells, the relative number of Heinz bodies being approximately 5 1 combined with a more coarse appearance of the Heinz bodies in cells from newborns This discrepancy between the two sets of experiments is a strong argument against the idea that a liability of HbF to oxidation is directly related to the liability to Heinz body formation.

There was a difference between HbF cells and HbA-cells of the newborns regarding Heinz body formation. HbA cells showed a somewhat smaller number of Heinz bodies in the mean. However the difference was not impressive 4.8 (in A-cells) versus 5.6 (in F-cells). HbA cells of the newborn therefore are not comparable with HbA-cells of an adult One is led to the conclusion that within the same compartment (the newborn red cell) the difference between HbF and HbA with regard to the action of acetyl phenylhydrazine is the same as could be seen in the experiments with haemolysates. However it must be stated that the mean cell age of HbF-cells in blood from newborns will be higher than that of HbA-cells; younger cells are less susceptible to Heinz body formation than are older cells.

In any case it seems well established that HbF plays a minor role only—if any at all—for the enhanced Heinz body formation in red cells of newborn infants. Since the amount of reduced glutathione has no decisive significance for this phenomenon either as shown by Tjon, there must be some other characteristic property of the red cells of newborns making them labile in this respect

Summary

Heinz body formation by incubation of blood with acetylphenylhydrazine was more pronounced in erythrocytes of newborns than in erythrocytes of adults the ratio being approximately 5 1 In haemolysates cord haemoglobin was denatured only slightly faster by acetylphenylhydrazine than was adult haemoglobin. By differentiating HbA-cells from HbF cells in blood smears from newborns with the acid elution technic it could be shown that Heinz body formation proceeded nearly as fast in erythrocytes containing HbA as in erythrocytes containing HbF

HbF therefore does not play a significant role for the enhanced Heinz body formation in red cells of newborn infants. Since the amount of reduced glutathione has no decisive significance either, there must be some other characteristic property of the red cells of newborns making them labile in this respect.

References

1. Betke, K. and Kleihauer, E.: Fetaler und bleibender Blutfarbstoff in Erythrocyten und Erythroblasten von menschlichen Feten und Neugeborenen. *Blut*, *4*: 241, 1958.
2. Betke, K., Greinacher, I. and Hecker, F.: Oxydation menschlicher und tierischer Oxyhämoglobine durch Kaliumferricyanid. *Arch exper Path Pharmak*, *229*: 207, 1956.
3. Betke, K., Greinacher, I., and Tietze, O.: Oxydation menschlicher und tierischer Oxyhämoglobine durch Natriumnitrit. *Arch exper Path Pharmak*, *229*: 220, 1956.
4. Gasser, C.: Die hämolytische Frühgeburtenanämie mit spontaner Innenkörperbildung. *Helvet Paediatr Acta*, *8*: 491, 1953.
5. Gröschner, E.: Unpublished investigations, personal communication.
6. Heubner, W.: Methämoglobin, Innenkörper der Erythrocyten und Anämie. *Klin Wschr*, *20*: 137, 1941.
7. Jandl, J. H., Engle, L. K. and Allen, D. W.: Oxydative hemolysis and precipitation of hemoglobin. I. Heinz body anemias as an acceleration of red cell aging. *J Clin Invest*, *39*: 1818, 1960.
8. Jung, F.: Über Heinzsche Körperchen. *Dtsch. Gesundh. Wes.*, *9*: 773, 1954.
9. Künzer, W. and Schutz, B.: Untersuchungen zur Heinzkörperbildung in Neugeborenenerythrocyten. 5. Mitt. Heinzkörperbildung an hämoglobinfreien Erythrocytenschatten. *Z Kinderheilk*, *77*: 96, 1956.
10. T[illegible], G. T.: Untersuchungen über Heinzkörperbildung und Glutathionstabilität in Erythrocyten von Neugeborenen. *Z Kinderheilk*, *84*: 484, 1960.
11. Willi, H.: Innenkörperbildung durch Elkosin und spontane Innenkörperbildung. *Schweiz Med Wschr*, *77*: 243, 1947.
12. Zinkham, W. H.: An in vitro abnormality of glutathione metabolism in erythrocytes from normal newborns. *Pediatrics*, *23*: 18, 1959.

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Erythropoietin Levels in Cord Blood as an Indicator of Intrauterine Hypoxia

by PER HAAVARDSHOLM FINNE

Increased levels of erythropoietin have been demonstrated in cord blood from infants with hemolytic anemia due to maternal Rh immunization. In severe cases erythropoietin has also been demonstrated in amniotic fluid [8 10]. These findings, together with the demonstration of erythropoietin in normal cord blood [11] show that erythropoiesis antenatally—at least in the last months of gestation—is regulated through erythropoietin.

It is generally accepted that the production of erythropoietin is stimulated by hypoxia, be it anemic or hypoxic. Increased erythropoietin levels should therefore be expected in cord blood of infants suffering from intrauterine hypoxic hypoxia. In some pathological pregnancies the foetus may be exposed to prolonged hypoxia e g in dysmature infants [1 23], preeclamptic pregnancies [12 23] and prolonged pregnancies [23, 24]. However there is some disagreement concerning the oxygen saturation of cord blood in prolonged pregnancies; some investigators have found normal oxygen saturation of cord blood in such pregnancies [7 14 19] and no difference in cord blood hemoglobin and red cell values between these infants and normal infants at term [3, 15 18].

The present paper reports investigations carried out in an attempt to answer the question. Are increased levels of erythropoietin found in cord blood from infants delivered after pregnancies in which the foetus is supposed to suffer an intrauterine hypoxic hypoxia?

Material and Methods

Cord blood was collected from 19 preeclamptic, 15 prolonged and 36 Rh immunized pregnancies, from infants of 8 diabetic mothers and from 13 premature infant weighing less than 2500 g in whom the prematurity seemed to be the only abnormality. As a control cord blood was studied from 14 infants who went through normal gestation and delivery with normal hemoglobin and reticulocyte values and normal placentas on macroscopic examination. In 3 cases amniotic fluid was collected *either by transabdominal puncture before* birth, or at delivery. The blood was collected from the umbilical cord and consisted of mixed arterial and venous blood. In some cases of Rh-immunization blood was withdrawn during the first part of exchange transfusions performed shortly after the birth. The blood samples were centrifuged immediately after collection, the plasma pipetted off and kept at −20°C until studied. The placentas were inspected in all but the Rh-immunized pregnancies. Gross patho-

TABLE I *Percentage Fe^{59} incorporation into red cells in polycythemic mice injected subcutaneously on two consecutive days with 0 5 ml plasma from normal infants born at term and from "normal" premature infants weighing less than 2500 g*

Figures in parentheses indicate number of recipient mice

Plasma from normal infants born at term					Plasma from premature infants weighing less than 2500 g.			
	Capillary blood							
Name	Hb(g)	Retics. ‰	Weight (g)	Fe^{59} uptake % ± s.e.	Name	Gest week	Weight (g)	Fe^{59} uptake % ± s.e.
Us.	20.8	13	3770	4.0 ± 2.7 (3)	Rw	32	1520	0.1 ± 0.02 (2)
Fl.	18.0	14	3060	1 7 ± 0.4 (4)	Th.	31	1680	[illegible]1 ± 1 1 (3)
Ha.	20.4	23	3770	2.7 ± 1.4 (3)	My	34	1320	0.8 ± 0.4 (4)
Mb.	20.8	53	4250	0.5 ± 0.1 (3)	Rl.	33	1960	0.1 ± 0.03 (2)
Gu.	18.0	24	4180	3.8 ± 2.0 (2)	Sn.	36	2490	2.0 ± 0.9 (5)
Pl.	20 0	26	3960	2.9 ± 1.5 (3)	Öb.	33	1760	1.3 ± 0 1 (3)
Bu.	20.0	33	3260	4.9 ± 1.2 (4)	Tf.	33	1600	0.6 ± 0.[illegible] (4)
Re.	19.4	25	4180	4.5 ± 3.6 (2)	Bö.	33	2350	0.9 ± 0.6 (3)
Rh.	20.4	21	2720	5.0 ± 1.9 (3)	Re	35	2460	1 9 ± 0.2 (3)
Ij.	20.0	53	2820	1 1 ± 0.5 (3)	St.	36	1870	2 4 ± 1.7 (2)
On.	19.4	76	3980	6.9 ± 3.4 (4)	An.	33	1670	1.7 ± 0.3 (2)
E	16.9	33	3280	2.8 ± 0 9 (4)	Kj.	36	2000	0.5 ± 0.2 (4)
Fm.	19.1	29	3520	2.8 ± 0.7 (3)	Ow	34	1740	0.3 ± 0.1 (3)
Dp.	17.5	27	3060	1.3 ± 0.6 (4)				
Mean ± S.E.				3.2 ± 0 4				1 1 ± 0.2

The difference between the two groups $t = 4.66$, $p < 0.001$

logical changes were recorded (infarction, low weight small circumference).

Abnormal clinical findings in the infants were also recorded. Discrepancy between length of gestation and birth weight, dry skin, and emaciation were regarded as signs of dysmaturity The dysmaturity was not graded.

The gestational age was calculated from the first day of the last menstrual period. Postmaturity was said to be present when the gestational age exceeded 42 weeks.

Patients with preeclampsia were divided into three groups: Mild, with moderate hypertension and no proteinuria, moderate with proteinuria and hypertension, and severe, in which marked proteinuria and hypertension were present. In some of the latter cases hypertension was so severe that signs of retinopathy had developed.

The hemoglobin values in the mothers were determined and all had values above 10.4 g.

Smears for reticulocyte counts in the normal infants were made from capillary blood and stained with Nile blue. 2000 cells were counted in each smear.

TABLE 2. *Percentage Fe^{59} incorporation into red cells in polycythemic mice injected subcutaneously on two consecutive days with 0.5 ml plasma from infants born more than 14 days after term*

Figures in parentheses indicate number of recipient mice.

Name	Fe^{59} uptake % ± s.e.	Comments
Lo.	46.4 ± 2.8 (4)	43 week, dysmature W 2920 g.
Ol.	22... ± 9.1 ( )	43 week, dysmature, placental infarction, W ·900 g.
Fr	19.3 ± 10.0 (2)	45 week, dysmature, W 3140 g.
Ba.	8.5 ± 2.3 (3)	44 week, W 4340 g
Sa.	8.0 ± 1 6 (3)	43 week, W 3940 g.
Pl.	6.4 ± 3.5 (5)	43 week, W 3330 g.
La.	1 4 ± 0.6 (4)	43 week, W 4..60 g.
Os.	7.2 (1)	44 week, W 4240 g
Joh.	1.3 ± 0.6 (4)	43 week, W 3160 g.
Fu.	1 1 ± 0.2 (3)	44 week, W 3430 g.
Be.	2.2 ± 0.5 (4)	43 week, W 3960 g. Dysmature?
Nl.	2.6 ± 1.0 (3)	43 week, W 3480 g.
Gu.	−.6 ± 1.8 (2)	42–43 week, W 3490 g.
Li.	0 1 ± 0.07 (3)	43 week, W 3610 g
Th.	0.8 ± 0.2 (2)	43 week, W 4100 g.

The erythropoietin in the plasma and amniotic fluid was determined by the use of the 72-hours Fe^{59} erythrocyte uptake in transfusion-induced polycythemic mice [8, 17]. Cord plasma was injected in doses of 0.5 ml on two consecutive days, and amniotic fluid in doses of 1 ml on two consecutive days. All injections were performed subcutaneously. A well scintillation counter was used (FH 4888 Friescke & Hoepfner Erlangen Bruch Germany) with a gamma sensitive crystal and with an efficiency of about 6.5 10^4 cp 0.1 microcurie of Cs^{137} at a background of 470–490 cpm.

Results

Table 1 shows the erythropoietin content in cord blood from normal infants born at term and without any complications. In the same table is shown the erythropoietin content in cord blood from "normal" premature newborn infants weighing less than 2500 g. It is seen that the erythropoietin levels are significantly lower in the premature group than in the normal group ($t = 4.66$, $p < 0.001$).

Table 2 shows the erythropoietin con

TABLE 3. *Percentage Fe^{59} incorporation into red cells in polycythemic mice injected subcutaneously on two consecutive days with 0.5 ml plasma (1 ml amniotic fluid) from infants born of preeclamptic mothers. Preeclampsia is divided into three groups according to the severity of the disease (severe, moderate, mild)*

Figures in parentheses indicate number of recipient mice.

Name	Fe^{59} uptake % ± S.E.	Comments
An.	38.8 ± 4.0 (3)	Severe, retinopathy, placental dysfunction, 42 week, infant cyanotic, dysmature, W 1910 g.
Jö.	29.1 ± 2.0 (2)	Moderate, placental infarction, 40 week, dysmature, W 2300 g.
Bö.	32.8 ± 2.3 (2)	Severe, placental infarction, 40 week, dysmature, W 2000 g.
An.	22.3 ± 4.7 (4)	Severe, placental dysfunction, death in utero.
Kr	[illegible] ± 3.4 (3)	Moderate, placental infarction, 41–42 week, dysmature, W 17?0 g.
Li.	18.9 ± 2.8 (4)	Severe, placental infarction, 35 week, W 1720 g
Lill.	15.0 ± 6.1 (3)	Severe, retinopathy, placental infarction, 35 week, W 1 60 g
Jo.	14.4 ± 1.9 (4)	Moderate, 44 week, dysmature, W 1000 g, death during delivery
Es.	12.9 ± 1.0 (2)	Severe, placental infarction, 34 week, W 1200 g.
Röb.	10.6 ± 1.9 (4)	Moderate, 40 week, W 3300 g.
Joh.	4.4 ± 1.1 (3)	Moderate, 40 week, W 3400 g
Kri.	4.1 ± 1.2 (3)	Moderate, 37 week, W 2020 g.
Uh.	4.1 ± 1.6 (3)	Moderate, placental infarction, 39 week, W 3400 g.
Fr.	3.3 ± 2.0 (4)	Moderate, 40 week, W 3290 g.
Eng.	[illegible].7 ± 1.6 (3)	Moderate, 40 week, W 3280 g
La.	2.3 ± 1.3 (3)	Moderate, 40 week, W 3240 g.
Ol.	1.6 ± 0.2 (2)	Mild, 40 week, W 3800 g
Be.	1.5 ± 0.5 (2)	Mild, 40 week, W 3? 0 g.
Mo.	1.2 ± 0.3 (3)	Severe, 42 week, W 400 g
Sv	0.4 ± 0.1 (4)	Moderate, 40 week, W 3200 g.

Amniotic fluid.

tent in cord blood from infants delivered more than [illegible] weeks after term. In some of these cases the erythropoietin levels are significantly higher than in normal cord blood (mean normal 3.2% + 2.5 S.D = 7.4%). The infants with the highest erythropoietin values showed signs of dysmaturity.

TABLE 4. *Percentage Fe^{59} incorporation into red cells in polycythemic mice injected subcutaneously on two consecutive days with 0.5 ml plasma (1 ml amniotic fluid) from infants born of diabetic mothers*

Figures in parentheses indicate number of recipient mice.

Name	Fe^{59} uptake ± S.E.	Comments
Da.	37.3 ± 4.1 (3)	Death in utero, 36 week, W 2810 g.
Pl.	28.8 ± 0.5 (5)	Death in utero, 34 week, W 680 g.
Wa.	11.9 ± [illegible] (4)	Death hours post partum, 38 week, W 3520 g.
Joh.	7.7 ± 1.4 (4)	36 week, W 3520 g.
Kr.	3.4 ± 0.8 (4)	36 week, W 3070 g.
La.	2.0 ± 0.8 (3)	36 week, W 3600 g.
Ga.	1.6 ± 0.3 (2)	37 week, W 3450 g.
Ha.	1.0 ± 0.5 (5)	36 week, W 3380 g.
Xl.	0.9 ± 0.4 ([illegible])	36 week, W 3900 g.
Gr.	0.5 ± 0.3 (4)	37 week, W 3800 g.

Amniotic fluid.

Table 3 shows the erythropoietin levels in cord blood from infants born of pre-eclamptic mothers. The highest levels are classified as severe. Some of the infants in this group also showed clinical signs of dysmaturity.

Table 4 shows the erythropoietin content in cord blood from infants delivered of diabetic mothers. The erythropoietin content in amniotic fluid is shown in two cases. In pregnancies where the foetus died, the amniotic fluid contained high levels of erythropoietin. As shown earlier there is a good correlation between the erythropoietin levels in cord blood and amniotic fluid [8]. Thus, increased erythropoietin in amniotic fluid indicates elevated levels in cord blood as well, though it was not determined in these two cases. In uncomplicated cases the erythropoietin levels in cord blood were normal or slightly elevated.

Table 5 shows the erythropoietin content in cord blood from infants with varying degrees of hemolytic disease of the newborn. It is seen that with increasing anemia the erythropoietin levels rise, and from a hemoglobin level of about 13–11 g it is likely to be significantly higher than in normal cord blood.

The data demonstrated in the tables are visualized in Fig. 1.

Discussion

The results show that increased erythropoietin levels may be found in cord blood

TABLE 5 *Percentage Fe^{59} incorporation into red cells in polycythemic mice injected subcutaneously on two consecutive days with 0 5 ml plasma from erythroblastotic infants The infants were divided into groups according to their capillary hemoglobin values.*

The mean and standard error of the mean (s.e.) for each group is given and so is also the statistical significance test bet een each group and the normal infants born t term Figures in parentheses indicate numbe of recipient mice.

Capillary Hb(g)	Name	Fe^{59} uptake % ± s.e.		Mean in each group % ± s.e.
5–7	Ha.	19.3 ± 2.0	(5)	26.0 ± 6.7
	Ho.	32.7 ± 2.3	(3)	0.005 > p > 0.001
7 9	Pa.	42.8 ± 5.0	(2)	
	Ba.	3.5 ± 0.3	(5)	
	Sk.	23.1 ± 2.1	(4)	
	Leu.	0.4 ± 0.1	(4)	20 2 ± 5 6
	Sn.	14.8 ± 1.3	(5)	0.01 > p > 0.005
	Hå.	21.8 ± 5.1	(5)	
	Dy	50.0 ± 3.0	(3)	
	Gum.	10.5 ± 4.5	(3)	
	Gu.	15.3 ± 3.7	(4)	
9–11	El.	10.4 ± 1.2	(4)	
	Pr	27 1 ± 2.5	(4)	
	Böe.	23.5 ± 0.7	(4)	14.1 ± 4.3
	Br	1.3 ± 0.3	(4)	0.025 > p > 0 020
	Joh.	2.7 ± 0.2	(4)	
	Pe.	26.8 ± 3.3	(6)	
	Ols.	7.5 ± 1.5	(4)	
11 13	So.	11 1 ± 3.7	(4)	
	To.	19.1 ± 7.3	(4)	
	Joh.	6.3 ± 1 1	(6)	
	My	3.3 ± 0 3	(3)	11 0 ± 3.1
	Hj.	1.9 ± 0.3	(2)	0 025 p > 0.020
	Bo.	12.9 ± 4 0	(4)	
	Pe.	23.7 ± 8.9	(3)	
13–15	J	0.6 ± 0 4	(3)	
	Er	25 0 ± 8 7	(3)	
	F	2 2 ± 0.7	(5)	
	Ml.	0 7 ± 0.3	(3)	
	Kr	15 0 0.3	(3)	3 1 ± 2.8
	U	4 1 ± 0.6	(6)	0.80 > p > 0.70
	La	0 4 0.1	(4)	
	Ju.	2 4 ± 0 9	(4)	
	St.	0.4 ± 0 3	(4)	
15–17	Nyh.	0 4 + 0.1	(4)	
	La.	2 3 0 6	(4)	
	He.	6.2 3 4	(3)	
	El	0.6 ± 0.3	(3)	2 0 ± 0.7
	Bör	2.4 ± 1 4	(4)	0.90 p 0.80
	Bjo.	2.2 0.9	(4)	
	Ol.	1 0 0.2	(3)	
	Am.	0.3 0.06	(4)	

Cord blood hemoglobin.

Table 3. (*continued*).

Capillary Hb(g)	Name	Fe^{59} uptake % ± s.n.		Mean in each group % ± s.e.
17–18	Ajo.	3.2 ± 0.8	(2)	
	Lo.	6.1 ± 1.3	(3)	
	Kv	3.9 ± 1.6	( )	4.4 ± 1.4
	Ml.	10.3 ± 2.3	(3)	0.50 > p > 0.40
	Ut.	0.9 ± 0.2	(2)	
	Go.	2.2 ± [illegible]0	(3)	
19–20	Bå.	[illegible]6 ± 0.6	([illegible])	
	Ja.	10.7 ± 4.7	(3)	
	Gu.	2.9 ± 0.6	(4)	
	Ku.	0.8 ± 0.3	(4)	3.3 ± 1.1
	Ev	3.3 ± 0.9	(4)	0.93 > p > 0.90
	Ho.	1.9 ± 0.2	(2)	
	Ev	1.3 ± 0.6	(3)	
	Ly	3.1 ± [illegible]5	(3)	

not only in anemic newborn infants but also in infants suffering from hypoxia due to other causes.

The erythropoietin levels in normal full-term newborns are significantly higher than in a group of "normal" premature infants weighing less than 2500 g. Some investigators have shown an increase in red cell and hemoglobin values with increasing age of the foetus as a result of a decrease in oxygen saturation of cord blood [23, 24]. However other investigators have not been able to confirm these findings [3, 7, 14, 15, 19]. The results of the erythropoietin investigations in these two groups seem to indicate that at term there is less oxygen available for the foetus compared with the demand, than at an earlier stage of the pregnancy. This, in turn, results in increased erythropoietin content in the blood. One must assume that the erythropoietin plays the same role in the regulation of red cell production in both groups. Some investigators postulate that in fetal life there is an early period in which the organism is unresponsive to adult erythropoietic regulatory mechanisms, i.e. the adult regulating factor erythropoietin acts only during the last period of intrauterine life [22]. However it should be pointed out that, in erythroblastotic infants, highly elevated erythropoietin levels have been found both in cord blood and amniotic fluid as early as the 32th week of gestation. However we do not know if erythropoietin acts and is demonstrable before this time. The influence of delivery on the erythropoietin levels can not be excluded, although the infants in both groups have gone through clinically normal deliveries. However it is unlikely that a normal delivery significantly interferes with the oxygen supply to the normal foetus [5, 20]. An eventual hypoxia caused by the delivery would have to last for several hours if the amount of erythropoietin produced should be in the range which could give a measurable rise in the plasma. It is therefore hard to explain the differences in the erythropoietin levels between the fullterm and the premature infants as caused by the labor itself.

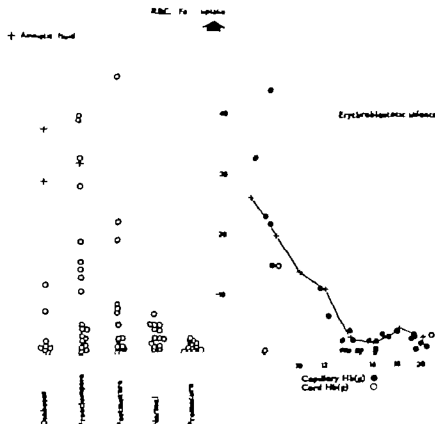


Fig. 1. The figure shows the Fe^{59} incorporation into red cells in polycythemic mice injected subcutaneously with plasma from infants belonging to the different groups investigated. The right part of the figure represents the erythroblastotic infants, these were divided into various groups according to their capillary hemoglobin values. Mean in each group is calculated and the curve is based on these calculations. The figure is based on the same data as presented in the tables.

In the group of prolonged pregnancies, some infants with markedly elevated erythropoietin content in cord blood were found, but in these cases the infants showed clinical signs of dysmaturity and in one case infarcts of the placenta were also present. It is not clear whether dysmaturity is caused by the prolonged gestational time or not. Sjöstedt *et al.* [21] point out that evidence of dysmaturity is already manifest at term. This indicates that prolonged pregnancy itself does not cause dysmaturity. If one excludes the infants with clinical signs of dysmaturity the other infants showed about the same erythropoietin content in cord blood as the normal fullterm infants did. It has been pointed out that the rise in hemoglobin and red cell values found by some investigators with increasing length of gestation could be caused by hemoconcentration [3, 4] and that only determinations of red cell mass would be conclusive. The same objections may be raised concerning the dysmature group, since the period of intrauterine deprivation which

the foetus has endured [9] tends to give a shift in extracellular fluid. Concerning the erythropoietin determinations, these objections are not valid. One must assume that the malnourished foetus will be less able to respond to hypoxia with raised erythropoietin production. In protein deprived rats the erythropoietin production is known to be significantly lowered [16].

The infants of preeclamptic mothers seem to show the greatest frequency of elevated erythropoietin levels in cord blood indicating the high risk of intrauterine hypoxia, especially in severe cases. As seen from Table 3 the incidence of dysmaturity and placental infarctions is high in this group. This is in accordance with the findings of other investigators [13]. The high erythropoietin levels in many of these infants fit in well with other reports [12, 23], in which reduced oxygen saturation of cord blood, elevated hemoglobin and red cell values indicating fetal hypoxia in many preeclamptic pregnancies were found. Though placental infarctions were present in many cases with increased erythropoietin levels, there are cases without gross placental changes, but with significantly elevated erythropoietin titers in cord blood. Other changes leading to fetal hypoxia may also occur in preeclampsia. Browne & Veall [_] found a reduction in maternal placental blood flow and Dixon & Robertson [6] showed obliterative changes in the uterine arterioles supplying the placental bed in preeclamptic patients.

In two infants of diabetic mothers who died in utero high erythropoietin levels were found in the amniotic fluid. This indicates that the foetus may have gone through a period of severe hypoxia before death. In the cases where the infants were in good clinical condition the erythropoietin level in cord blood was normal or slightly elevated. Since all the infants were delivered four weeks before term it is not quite correct to compare these erythropoietin levels to those found in normal infants born at term. It may be supposed that even in clinically well infants of diabetic mothers some degree of reduced oxygen supply compared to the demand may occasionally be present, although in most uncomplicated cases there is no evidence of hypoxia. The increased erythropoietin levels found in some cases is in agreement with the work of Berglund & Zetterström [1], who found lowered oxygen content and elevated levels of nucleated red cells in a group of infants born of diabetic mothers compared with normal newborns.

As shown in Table 5 and Fig. 1 the erythropoietin levels increase with increasing severity of the anemia of the foetus, and from a hemoglobin level of about 13–11 g it is, in most cases, significantly higher than in normal cord blood ($0.025 > p > 0.0?$)

Anemic erythroblastotic infants have a pronounced extramedullary erythropoiesis. It may therefore be concluded that erythropoietin also stimulates extramedullary red cell production, this means that in the foetus erythropoietin may stimulate extramedullary as well as intramedullary stem cell to differentiate into red cells.

In conclusion one may say that intrauterine hypoxia, anemic or hypoxic, at least in the last part of intrauterine life, may cause an increase in erythropoietin production. Increased erythropoietin levels in cord blood in different pathological

pregnancies indicates that various causes of hypoxia may be present. Elevated cord blood erythropoietin levels suggests that the placental reserve is reduced, and any factors compromising the oxygen supply to the foetus during labor will be associated with an increased risk of foetal asphyxia and death. In erythroblastosis, hypoxic hypoxia may exaggerate the anemic hypoxia and give an increase in erythropoietin production beyond that which corresponds to the anemia, and this may in part account for the variance in erythropoietin levels found at the same hemoglobin values in different infants.

In a group of full term infants higher erythropoietin levels were found than in a group of "normal premature weighing less than 2500 g. The most likely explanation of this is that at term there is less oxygen available compared with the demand than in an early stage of gestation.

Summary

The erythropoietin content in cord blood was determined from normal full term infants, from infant orn more than 2 weeks after term from ifants of preeclamptic, diabetic and Rh immunized mothers. The group of normal infants showed higher erythropoietin levels than the premature group. Highly elevated erythropoietin levels were found in some infants in the preeclamptic, the postmature group and in the infants of diabetic mothers. The greatest frequency of increased erythropoietin levels were found in the preeclamptic group. The infants with the greatest rise in erythropoietin content often showed clinical signs of dysmaturity. Erythroblastotic infants seemed to show a rise in erythropoietin levels when capillary hemoglobin fell below 12–11 g. The results indicate that both anemic and hypoxic hypoxia may give an increase in erythropoietin content and that erythropoietin is a stimulating factor for red cell production in fetal life, at least in the last months.

References

1. Bergqvist, G. and Zetterström, R.: Infants of Diabetic Mothers. I. Foetal Hypoxia in Maternal Diabetes. *Acta Paediat Scand*, 43: 368, 1954.

2. Browne, J. C., McClure and Veall, N.: The maternal placental blood flow in normotensive and hypertensive women. *J Obstet Gynaec Brit Emp*, 60: 141, 1953.

3. Cook, C. D., Brodie, H. R. and Allen, D. W.: Measurement of fetal hemoglobin in newborn infants. *Pediatrics* 20: 272, 1957.

4. Dawkins, M. J. R., Martin, J. D. and Spector, W. G.: Intra-partum asphyxia. *J Obstet Gynaec Brit Emp*, 68: 604, 1961.

5. Dawes, R.: Anaerobic metabolism in the human fetus. *Amer J Obstet Gynec*, 89: 241, 1964.

6. Dixon, H. G. and Robertson, W. B.: A study of the vessels of the placental bed in normotensive and hypertensive women. *J Obstet Gynaec Brit Emp*, 65: 803, 1958.

7. Clifford, T. N., Koerf, S. T. and Morley, G. W.: Fetal effects of prolonged pregnancy. *Amer J Obstet Gynec*, 85: 701, 1963.

8. Finne, P. H.: Erythropoietin Levels in the Amniotic Fluid, Particularly in Rh-immunized Pregnancies. *Acta Paediat Scand*, 53: 269, 1964.

9. Gruenwald, P.: Chronic Fetal Distress and Placental Insufficiency. *Biol Neonat*, 5: 215, 1963.

10. Halvorsen, S. and Finne, P. H.: Transfer of Erythropoietin to Amniotic Fluid in Rh immunized Pregnant Women. *Brit Med J*, I: 1132, 1963.

11. Halvorsen, S.: Plasma Erythropoietin Levels in Cord Blood and in Blood during the First Weeks of Life. *Acta Paediat Scand*, 52: 425, 1963.

12. KYANK, H. and EGGERT L.: Determination of pH values in the Umbilical Cord Blood of Newborn Infants Delivered by Women with Toxemia of Late Pregnancy. *Biol Neonat* 50, 1963.
13. LITTLE, W. A.: Placental Infarction. *Amer J Obstet Gynec, 15* 109, 1960.
14. MACKINNEY L. G. GOLDBERG, D. EHRLICH, F. E. and FREYMANN K. C. Chemical analyses of blood from the umbilical cord of the newborn: relation to fetal maturity and perinatal distress. *Pediatrics, 1* 535, 1958.
15. MARKS, J. GAIRDNER, D. and ROSCOE, J. D.: Blood formation in infancy. Part III Cord blood. *Arch Dis Child, 30* 117 1955.
16. REISSMANN K. R.: Protein Metabolism and Erythropoiesis. I. The Anemia of Protein Deprivation. *Blood, 23*, 137 1964.
17. ROSSE, W. F. WALDMANN, T. A. and HOUSTON, D. L.: Erythropoietin Assay Using Iron 59 Incorporation into Blood and Spleen of the Polycythemic Mouse. *Proc Soc Exp Biol Med, 109* 836, 196_.
18. ROOTH, G. and SJÖSTEDT, S.: Haemoglobin in cord blood in normal and prolonged pregnancy. *Arch Dis Child, 32*, 91 1957
19. ROOTH G. and SJÖSTEDT S.: Oxygen saturation in the umbilical vessels of the human foetus in normal and prolonged pregnancy. *Acta Obstet Gynec Scand, 36* 374, 1957
20. ROOTH, G.: Foetal Respiration. *Acta Paediat Scand, 52* 22, 1963.
21. SJÖSTEDT S., ENGLESON G. and ROOTH, G.: Dysmaturity. *Arch Dis Child, 33* 123, 1958.
22. STOHLMAN F. JR., LUCARELLI G. HOWARD, D., MORSE, B. and LEVENTHAL, B.: Regulation of erythropoiesis XVI. Cytokinetic patterns in disorders of erythropoiesis. *Medicine, 43* 651 1964
23. WALKER, J. and TURNBULL, P. N.: Haemoglobin and red cells in the human foetus and their relation to the oxygen content of the blood in the vessels of the umbilical cord. *Lancet, II* 31_, 1953.
24. WALKER, J.: *Anemia of the Infant.* Blackwell Sci. Publ., Oxford 1953, p. 136.

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A Study of Immunoglobulins in the Blood Serum of Infants with Interstitial Plasma Cellular Pneumonia

by M. KOLTAY and M. ILLYÉS

Interstitial plasma cellular pneumonia (i.p.) was first described thirty years ago however it is only since 1945 that it has been studied in detail. Most authors now agree that the causative agent of this pneumonia, with characteristic clinical symptoms and X ray picture is the Pneumocystis carinii [11 23, 37]. Initially i.p. seemed to occur only on the European Continent. Later however it was described in England and America [1 3, 4 5 6, 1., 20]; however in these cases the pathology and course of the disease showed some peculiar features, the most prominent being a significant hypogammaglobulinemia [7 9 11 12, 15 16 20 25 27 28 30 31 33] As minimal data is available concerning the immunoglobulins of the infantile ip cases in Europe such examinations were made on our own patients, employing immunological methods.

Material and Methods

Clinical material

Examinations were carried out on a total of 43 infants, 4 to 20 weeks old, with a clear clinical diagnosis of ip.; in 14 patients the diagnosis was further supported by the demonstration of the causative agent in the secretion taken from the trachea [36]. Three-quarters of our patients were prematures and with the exception of two, they were treated with pentamidine isothionate [32] from the time the diagnosis was established. Clinically symptomless condition, mild, medium intensity and grave symptoms were distinguished and registered from ø to + + + according to an empirical scale. The degree of gravity of the roentgenological findings was marked according to the scheme used in our clinic, ranging from I to V of which V meant the gravest alteration [23].

Laboratory methods

The total protein of the blood serum was determined by the biuret-method [13]. The paper electrophoresis was made in the usual way [16]. Acid fuchsin was employed to visualize the protein fractions [29], and the quantitative evaluation was made by elution. Gammaglobulin concentration, calculated to absolute value on the base of the total protein content and percentage of the gammaglobulin fraction, was given in mg/100 ml.

In the immunoelectrophoretic study Scheidegger micromethod [34] was used. An immunoelectrophoresis of mixed standard serum taken from adults, was always made on the same slide as the patients serum samples. The slides were evaluated after 24 and after 48 hours of immunodiffusion, comparing the serum to be examined with the control. In our experimental conditions, beta 2A (IgA) and beta 2M

Ta 1 *Behaviour of immunoglobulins in infants suffering from interstitial plasma cellular pneumonia.*

Age (weeks)	No. of cases	Clinical signs				X-ray picture					γ-glob mg (per cent) (Normal)	Immunoglobulins (immunoelectrophoresis)									Recovered	Deceased
												γ			β_2A			β_2M				
		Ø	+	++	+++	I	II	III	IV	V		→	↘	↗	0	↘	↗	0	↘	↗		
3–8	23	11	8	4	—	1	1	14	7	—	532±139	1	22	—	22	1	—	20	—	3	23	—
9–1	10	1	2	1	6	—	—	3		5	716±418	1	5	4	7	—	3	3	—	7	8	2
13–20	10	—	—	1	9	—	—	1	1	8	793±397	4	3	3	8	—	2	—	—	10	9	1

The normal concentration of immunoglobulin is shown by an arrow in horizontal position. Arrow showing downwards means reduction, arrow showing upwards means elevation of the corresponding immunoglobulin. 0 means that the corresponding immunoglobulin could not be demonstrated by immunoelectrophoresis.

(IgM) immunoglobulins appeared in the control serum only after 48-hour diffusion; their earlier appearance indicated a rise in the level of the respective immunoglobulin. Concerning the quantitative conditions of gammaglobulin (IgG) in immunoelectrophoresis, approximate conclusions were drawn from the length and width of its precipitation line

A semiquantitati assessment of the three immunoglobulins was made by a micro-modification of Ouchterlony double gel-diffusion method, depositing the antigen containers in a rosette from [26]. In immunodiffusion studies, polyvalent anti human horse immune serum (Human" Vaccine Producing and Research Institute, Budapest Hungary), and immune sera prepared in rabbits, reacting specifically with the human immunoglobulins, were used.

Results

In our material three groups of patients could be distinguished on the basis of age, clinical symptoms and X ray findings (Table 1).

In the first group consisting of 23 infants, the clinical and/or radiological symptoms of the ip appeared during the first two months of life and the disease could be considered on the whole as mild or of medium gravity In these patients the gammaglobulin level—in compliance with the physiological hypogammaglobulinemia—was generally low By immunoelectrophoresis, the other two immunoglobulins could be demonstrated only in some of the cases this finding however cannot be considered as pathological in infants less than half year of age In the blood serum of three infants of this group the concentration of beta 2M immunoglobulin was found to be moderately elevated.

The patients of the second and third group, each consisting of 10 infants, showed analogy to each other in numerous respects. In these infants the first symptoms appeared at the end of the third month of life or later were quite pronounced already at the beginning of the illness, and in most of the cases, the disease had a grave course Examining immunoelectrophoretically the sera of these patients—with the exception of three infants,

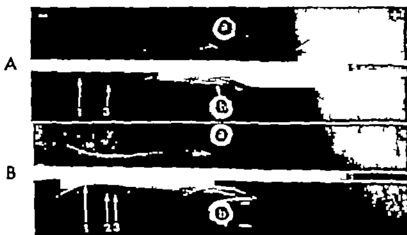


Fig. 1. Immunoelectrophoretic pattern of blood serum of infants suffering from interstitial plasma cellular pneumonia. Time of immunodiffusion: 24 hours. *a*, Control sera; *b* sera of infants suffering from grave form of ip.; *as*, polyvalent anti-human horse immunserum. *1* gammaglobulin; *2*, beta 2A immunoglobulin; *3*, beta 2M immunoglobulin. On slide A, an accentuation of the beta 2M precipitation line and on slide B, the accentuation of the precipitation lines of all the three immunoglobulins can be observed. In control sera, the beta 2A and beta 2M lines are not shown, they appear normally in the conditions used only after 48 hours of immunodiffusion.

who did not yet complete the third month of life—a pronounced elevation of the beta 2M immunoglobulin was striking (Fig. 1A). The other two immunoglobulins behaved variably: in some of the cases a decrease and in other cases an elevation could be noted, in two patients the increase of all the three immunoglobulins could be demonstrated (Fig. 1B). In a few cases the serum of infants suffering from grave ip. could be examined repeatedly by immunoelectrophoresis during the course of the illness: the results of such an examination are shown in Table 2.

In the blood serum of 17 patients

Table 2. *Variations of immunoglobulins in the blood serum of the infant B. L. 2½ months old suffering from grave interstitial plasma cellular pneumonia.*

Date of exam. 1963	Total serum protein	γ-glob. rel.	γ-glob., mg % (paperelectrophoresis)	Immunoglobulins (immunoelectrophoresis) γ	β_2A	β_2M	Clinical symptoms	X-ray picture
21.6	7.2	16.8	1200	↗	→	↗	+ + +	V
1.7	6.3	26.3	1760	↗↗	→	↗↗	+ + +	V
18.7	7.0	22.0	1840	↗↗	↗	↗↗	+ +	IV V

The normal concentration of immunoglobulin is shown by an arrow in horizontal position. Single arrow showing upwards means elevated concentration, double arrow showing in the same direction means strongly elevated concentration of the immunoglobulin.

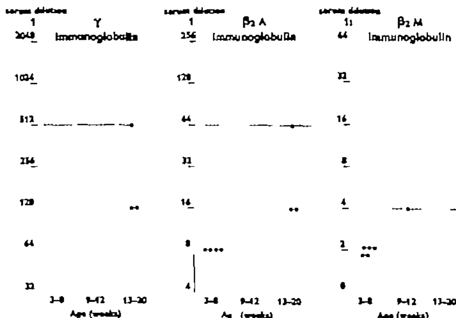


Fig. 2. Semiquantitative assay of immunoglobulins in blood serum of infants suffering from interstitial plasma cellular pneumonia, using a micro-modification of Ouchterlony's double-diffusion method. On the vertical axis of the diagram the serum dilutions, and on the horizontal axis, the age-groups are shown. The dotted lines show values found in pooled adult control sera.

suffering from ip. of variable intensity the concentration of the three immunoglobulins could be studied by the semi quantitative micro gel-diffusion method which is more sensitive than immunoelectrophoresis. The data thus obtained (Fig) were in agreement with those of our immunoelectrophoretic study and showed distinctly that when the ip appears in infants at the end of the first trimenon or later the rise in the concentration of the beta 2M immunoglobulin may be rather marked.

In our material, no conclusion could be drawn on the basis of the behaviour of the immunoglobulins, concerning the prognosis or the gravity of the illness. Three of our patients died (Table 1)· in one all the three immunoglobulins were elevated, whereas in the other two only the beta 2M immunoglobulin showed elevation.

Nor did the examination of the immunoglobulins aid in the diagnosis of the ip. because on the one hand, in the mild cases, no characteristic changes could be found and on the other the increase of the beta 2M immunoglobulin may appear in connection with other diseases in infancy and consequently cannot be considered as specific to the ip.

Discussion

The epidemiological features of ip the characteristic age-factor and the fact that the disease appears almost without exception in prematures or in dystrophic, debilitated infants, suggested, before the recognition of the causative agent, that diminished defensive capacity of the organism may play some role in the establishment of the infection [23] Because

prematures possess less gammaglobulin than mature newborns, the systematic administration of gammaglobulin in the prophylaxis of ip in premature infants had been suggested already in 1950 the results of such prophylaxis, however were not conclusive [10] Having recognized the antibody-deficiency syndromes [17], the question of the connection between gammaglobulin level and ip. was raised in consequence of the cases observed in England [4 20] and in America [12] wherein the concentration of serum-gammaglobulin was strikingly low Similar observations were reported from these countries in later years also [1 5 6 7 8 9 11 16, 25 27 28 30 31 33] In some cases lymphocytopenia was found [1 5 30] in addition to the hypogammaglobulinemia Recently ip. cases in connection with the Wiskott Aldrich syndrome associated with immunological anomalies, were also observed [8 38]

In Europe—though the incidence of the disease is the highest—rare and inconclusive examinations have been carried out concerning the immunoglobulins of ip. patients. An earlier study in our clinic, examining the phagocyte activity of leukocytes in infants suffering from ip showed, by paper electrophoresis, elevated gammaglobulin levels, as compared to controls of the same age [21] Other workers, using the same method, found hypogammaglobulinemia in patients 6–8 weeks old, in ip patients of older age however an increase of gammaglobulin concentration was noted [32].

Individual immunoglobulins of patients suffering from ip have not been systematically studied with immunoelectrophoresis or immunodiffusion and in this respect only sporadic observations are available English authors demonstrated, beside hypogammaglobulinemia a very considerable rise of the beta 2M immunoglobulin in a ten-month-old infant suffering from ip. [27] In another infant two months old, observed in Finland, concomitantly with ip. a marked rise in beta 2M immunoglobulin level could be demonstrated contemporaneously with normal levels of the other two immunoglobulins [4]. American authors found significantly elevated beta 2M immunoglobulin values in a 1½ year-old child, suffering from a partial antibody deficiency syndrome; the infant died, and at autopsy ip. could be dedemonstrated. It seems, however that in this case the rise of the beta 2M immunoglobulin preceded the appearance of ip [19]

The study of the problem and the evaluation of findings are rendered difficult by the fact that ip. occurs generally in very young infants, when the level of immunoglobulins and antibodies may be very low even under physiological circumstances. Further it is also possible that the ip cases observed in Europe, England and America are not quite identical.

The Pneumocystis carinii considered as the causative agent, can be demonstrated both in overseas and in European ip cases, together with the characteristic foamy material filling the alveoli. The interstitial infiltration of the lung in the European cases, however contains plasma cells in such a significant number that the descriptive term plasmacellular" figures in the original name of the illness; as opposed to this in the cases described in America and England the interstitial

infiltration is for the most part due to lymphocytes [15 16] Serologically the identity of European and oversea cases could not be demonstrated [14] moreover the ip described in America occurs frequently in patients older than 6 months, and its prognosis seems to be even more unfavourable than in the European cases [17]

In our study we examined systematically the immunoglobulins of the blood serum of infants of different age suffering from ip of various intensity using immunological methods In no case did we find a complete lack of immunoglobulins, i.e. their decrease exceeding the physiological limit whereas in infants suffering from severe forms of ip and older than two months of age elevated beta 2M immunoglobulin levels could be demonstrated with high frequency

Our data indicate that—although the predisposing role of the hypogammaglobulinemia cannot be excluded—there is no direct correlation between the ip cases observed in our country and the antibody deficiency syndromes Indirectly our data support the opinion that the European and oversea ip cases are not entirely identical. They also indicate that a transient insufficiency in the production of immunoglobulins cannot furnish sufficient explanation for the evolution of the grave forms of the disease, and that in this respect other aspects of the immunologic defence mechanisms of such patients should be studied too

The behaviour of the immunoglobulins in ip on the basis of our experience does not render it possible to draw valid diagnostic or prognostic conclusions. The accumulation of beta 2M immunoglobulin frequently found in infants suffering from ip. suggests that the infant reacts to the infection with the causative agent of the disease firstly by the production of antibodies of this type This interpretation is in agreement both with the observation that the antibodies against protozoons are often macroglobulins and that the infant easily produces antibodies of the macroglobulin type [* 35]

Summary

The behaviour of the three immunoglobulins (gammaglobulin, or IgG beta 2A globulin, or IgA and beta 2M globulin, or IgM) has been examined, using paper electrophoresis and immunological methods, in the blood serum of 43 infants suffering from interstitial plasma cellular pneumonia The level of the immunoglobulins was generally found to be decreased in those cases, where the symptoms of the disease were mild or of medium intensity and appeared in infants between 4–8 weeks of age i.e in the period of the physiological hypogammaglobulinemia.

If however the disease caused grave symptoms and was manifested in older infants, an increase of beta 2M immunoglobulin was found to be characteristic in some cases, the elevation of all the three immunoglobulins occurred.

Our observations suggest that in interstitial plasma cellular pneumonia of infants antibodies of the beta 2M type must play an important part, but that there is no direct correlation between the common antibody deficiency syndromes and the infantile interstitial plasmacellular pneumonia occurring in this country

References

1 ALLIBONE, E. C., GOLDIE, W. and MARMION, B. P.: Pneumocystis pneumonia and progressive vaccinia in siblings. *Arch. Dis. Child.*, *39* 26, 1964.

2. ALTEMEIER, W. A. and SMITH, R. T.: Immunologic aspects of resistance in early life. *Ped. Clin. N. Amer. 12* 663, 1965.

3. BERDNIKOFF, G.: Fourteen personal cases of Pneumocystis carinii pneumonia. *Canad. M. A. J. 80* 1, 1959.

4. BIRD, T. and THOMSON, J.: Pneumocystis Carinii pneumonia. *Lancet, I* 59, 1957.

5. BOUTON, J., MAINWARING, D. and SMITHELLS, R. W.: BCG dissemination in congenital hypogammaglobulinaemia. *Brit. Med. J. I* 1512, 1963.

6. BURKE, E. C., BROWN, A. L. and WEED, L. A.: Pneumocystis Carinii pneumonia. Report of a case in infant with hypogammaglobulinemia. *Proc. Staff Meet. Mayo Clin.*, *162*, 1962.

7. BURKE, B. A., KROVETZ, J. L. and GOOD, R. A.: Occurrence of pneumocystis Carinii pneumonia in children with agammaglobulinemia. *Pediatrics 28* 196, 1961.

8. DALLOZ, J. C., CASTAIN, Y. NEZELOFF, C. and SELIGMANN, M.: Paraprotéinémie transitoire de type gamma. Observation chez un nourrisson atteint du syndrome d'Aldrich. *Presse Méd.*, *73* 1341, 1965.

9. ERBERLING, E. W. and COHEN, F.: Pneumocystis Carinii pneumonia. Report of a case from Southwestern United States. *Pediatrics, 21* 345, 1958.

10. FREUDENBERG, E. and TOBLER, W.: Die interstitielle Pneumonie der Frühgeburten. *Ann. Paed.*, *175* 186, 1950.

11 GERRARD, J. W.: Pneumocystis pneumonia. *Ped. Clin. N. Amer. 5* 237, 1958.

12. GERRARD, J. W. and MOORE, D. F.: Interstitial plasma cell pneumonia due to Pneumocystis Carinii. *Canad. M. A. J. 76* 290, 1957.

13. GLEISS, J. and HITZENBERG, K.: Zur fraktionierten kolorimetrischen Bestimmung der Serumproteine im klinischen Laboratorium. *Z. ges. exp. Med.*, *116* 500, 1951.

14 GOETZ, O.: Serologische Befunde interstitieller Pneumonien aus den Vereinigten Staaten. *Arch. Kinderheilk.*, *170* 60, 1964.

15. GOOD, R. A., BRIDGES, R. A. and CONDIE, R. M.: Host-parasite relationships in patients with dysproteinemias. *Bact. Rev. 24* 115, 1960.

16. GOOD, R. A., KELLY, W. D. and GABRIELSEN, A. E.: Studies of the immunologic deficiency diseases: agammaglobulinemia, Hodgkin disease and sarcoidosis. *In* Grabar, P. and Miescher, P. (ed.): *II. Internat. Symp. of Immunopathology.* Schwabe, Basel and Stuttgart, 1962. p. 353–384.

17 GOOD, R. A., KELLY, W. D., RÖTSTEIN, J. and VARCO, R. L.: Immunological deficiency diseases: agammaglobulinemia, hypogammaglobulinemia, Hodgkin disease and sarcoidosis. *Progr. Allergy 6* 187, 1962.

18. GRASSMANN, W., HANNIG, K. and KNEDEL, M.: Über ein Verfahren zur elektrophoretischen Bestimmung der Serumproteine auf Filterpapier. *Dtsch. med. Wsch. 77* 333, 1951.

19. HONG, R., SCHUBERT, W. K., PERRIN, E. V. and WEST, C. D.: Antibody deficiency syndrome associated with beta₂ macroglobulinemia. *J. Pediat.*, *61* 831, 1962.

20. HUTCHINSON, J. H.: Congenital agammaglobulinemia. *Lancet, I* 844, 1955.

21 IVÁDY, J., DUX, E. and ILLYÉS, M.: Untersuchungen über die Phagocytosebereitschaft der Leukocyten von an interstitieller Pneumonie erkrankten Säuglingen. *Z. Kinderheilk.*, *85* 378, 1961.

22. IVÁDY, J., PÁLDY, L. and UNGER, G.: Weitere Erfahrungen bei der Behandlung der interstitiellen plasmacellulären Pneumonie mit Pentamidine. *Monatsschr. Kinderheilk.*, *111* 297, 1963.

23. KOMÁRIK, B., TIBOLDI, T., TÖRÖK, J., WALTNER, K., DIÓSSZILÁGYI, G., PÁLDY, L. and KOLTAY, M.: Die interstitielle Pneumonie der Frühgeborenen. *Acta Med. Acad. Sci. Hung. 5* 371, 1954.

24. KOUVALAINEN, K., HJELT, L. and WASZ-HÖCKERT, O.: Marked elevation of beta 2M globulin in a patient with interstitial plasma cell pneumonia. *Acta Paediat. Scand.*, *53* 485, 1964.

25. KRAMER, R. J., CIRONE, V. C. and MOORE, H.: Interstitial pneumonia due to Pneumocystis carinii cytomegalic inclusion disease and hypogammaglobulinemia occurring simultaneously in an infant. A case report with necropsy findings. *Pediatrics 29* 816, 1962.

26 MANCINI, W.: Slide gel diffusion precipitin test. *Nature*, *181* 1209, 1958.

27 MARSHALL, W. C., WESTON, H. J. and BODIAN, M.: Pneumocystis Carinii pneumonia and congenital hypogammaglobulinemia. *Arch. Dis. Childh.*, *39* 18, 1964.

28. McKAY, E. and RICHARDSON, J.: Pneumocystis Carinii pneumonia associated with hypogammaglobulinemia. *Lancet, II* 713, 1959.

29 NOWOTNY, A.: Über die Anwendung der Elektrophorese auf Filterpapier. *Acta Physiol. Hung. 3* 469, 1952.

30. ROSEN, F. S., J. B. MILLER, R. H. ARAN...

V M. and PEARSON H. A.: Successful treatment of pneumocystis Carinii pneumonia in a patient with congenital hypogammaglobulinemia. *New Engl J Med*, *273* 704, 1965.

31. ROGERS, T and HARDIN, M.: Pneumocystis Carinii pneumonia associated with hypogammaglobulinemia responding to pentamidine *Lancet*, *I* 1042, 1964.

32. RÖVER, G Über die Serumeiweissverhaltnisse bei fruhgeborenen Kindern unter besonderer Berucksichtigung der plasmacellularen Pneumonie. *Z Kinderheilk*, *73* 601, 1963.

33. RUSSELL, J G Pneumocystis pneumonia associated with agammaglobulinemia. *Arch Dis Child*, *34* 338, 1959.

34. SCHEIDEGGER, J J Une micro-méthode de l immuno-électrophorèse. *Int Arch Allergy*, *7* 103 1955.

35. SMITH, R. T., EITZMAN D V and MILLER, B. E.: Qualitative differences in the immune response of infants and adults receiving salmonella vaccines. *J Clin Invest*, *39* 1029 1960.

36. TÓTH, G BALOG, E. and BÉLAY M.: Demonstration of the etiologic agent of interstitial plasma cellular pneumonia in tracheal secretions. *Acta Paed Hung* *7* 25, 1966.

37. TEUCKENBRODT H HÖVELS, O., SULZBECK, H. and VIVELL, O Zu Klinik und Epidemiologie der interstitiellen Pneumonie. *Med Klin*, *60* 1269 1965

38. WEINTRAUB, H. D and WILSON W J.: Pneumocystis Carinii pneumonia in Wiskott-Aldrich syndrome. *Amer J Dis Child* *108*. 198, 1964.

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Patent Ductus Arteriosus Associated with Pulmonary Hyperten ion

Postoperative Changes in the Hemodynamics and in the Electrocardiogram

by LEENA TUUTERI and KRYSTYNA BORKOWSKA[1]

Pulmonary hypertension is reported to occur in 10 to 15 per cent of cases with patent ductus arteriosus [14 19 26] The hypertension is mainly determined by two factors: increased pulmonary blood flow and increased pulmonary vascular resistance. If the elevated pulmonary arterial pressure is hyperkinetic in nature [27] the hemodynamics usually become normal or nearly normal after elimination of the shunt. On the other hand, patients with pulmonary hypertension and elevated pulmonary vascular resistance present special problems, because the operative risk is higher and the effect of the operation on the hemodynamics is less predictable than in uncomplicated cases.

The purpose of this paper is to present follow up studies of 4 children operated upon for patent ductus arteriosus with pulmonary hypertension.

Material and Methods

From 1938 to 1962, 450 children with patent ductus arteriosus were operated upon in Children Hospital, Helsinki. Moderat to severe pulmonary hypertension was present in 45 of the operated cases. There were 3 postoperative deaths in the cases with pulmonary hypertension. Postoperative catheterization was not routinely performed before 1962. The present material includes 24 cases in which sufficient pre- and post operative data were vailable.

The age f the children t operation ranged from 10 months to 14 years. There were 16 girls and 8 boys. A venous catheterization was performed in all cases before surgery and at least once postoperatively All patients had a left to right shunt, a systolic pressure in the pulmonary artery of 60 mm Hg or more and a mean pressure of t least 30 mm Hg. In one case a mild aortic stenosis was present as a complicating lesion.

The follow-up time from operation to the last catheterization ranged from 6 months to 4½ years. The catheterization was performed under light sedation. Pressures were measured with an Elema strain gauge pressure transducer and recorded with an Elema directwriting Mingograph 24 pparatus. The zero point was taken at 3 cm below the sternal angle. Prior to operation the systemic pressure was measured in the descending aorta, which could be entered through the ductus in all cases. Postoperati ely the pressure was determined by puncture of the femoral or brachial artery

The oxygen saturation of the blood was determined with a Brinkman hemoreflector The ratio between the systemic and pulmonary arterio- enous O -difference was calculated.

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Electrocardiograms including the leads I II III, AVR, AVL, AVF V V and V were recorded at all examinations. The rate, rhythm, electrical position and frontal axis of the heart, the duration and height f the P waves in leads II and V_1, the amplitudes of R, S and T waves in all leads, the R/S ratio in leads V and V and the duration of the P R interval and S-T segment were studied according to standard procedures. For the evaluation of ventricular hypertrophy the criteria given by Keith and associates [14] were used.

Lung biopsies were obtained at peration in 11 cases, usually from the lingula.

Results

Cli ical data

In 23 patients the ductus remained closed after operation. In one case (case 8) recanalization occurred and a small left to right shunt with normal pulmonary arterial pressure was present at the time of the last catheterization. The ductus was subsequently closed at a second operation.

In 23 cases the exercise tolerance was decreased before operation, an improvement occurred in all cases. One child had l exercise tolerance before surgery and remained asymptomatic.

Before operation 13 patients had a continuous murmur 8 patients a systolic and early diastolic murmur and 3 patients a pure systolic murmur In all cases the second pulmonary sound was accentuated. Postoperatively the auscultatory findings were normal in 18 cases. Four children had a soft grade 2–3/6 systolic murmur and five children a diastolic murmur in the pulmonary area, probably caused by mild pulmonary incompetence The child with aortic stenosis had a grade 5/6 systolic murmur in the second right intercostal space.

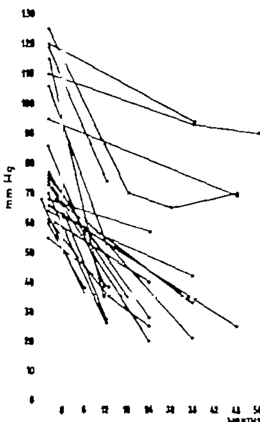


Fig 1 Pulmonary arterial systolic pressure before and after operation.

The size of the heart, which was preoperatively increased in all cases, decreased in every case

Hemodynamics

The pre- and postoperative data are presented in Table 1 Following surgery the pulmonary arterial systolic pressure decreased in all cases (Fig. 1) The pulmonary arterial diastolic pressure decreased in 23 cases, in 19 10 mm Hg or more. The postoperative pulmonary systolic and diastolic pressure tended to be lower in the younger children and in the cases with large shunts (Tables 1 and 2) In 6 cases the branches of the pulmonary artery

TABLE 1. *24 cases of patent ductus arteriosus with pulmonary hypertension. Pre- and postoperative data.*

No.	Age at op. in years	Systemic pressure preop.	Systemic pressure postop.	Pulmonary pressure preop.	Pulmonary pressure postop.	Follow up time in months	AVDs/AVDp	Ekg preop.	Ekg postop.
1	14	135/80	115/80	[illegible]5/40	58/30	48	3	CVH	N
2	13	115/55	12[illegible]/90	110/85	60/40	84	1.9	RVH	RVH
3	1	160/43	90/68	[illegible]/19	42/8	36	3.5	CVH	N
4	11	130/55	1[illegible]4/65	120/50	94/40	36	1.6	CVH	RVH
5	10	[illegible]/46	114/63	77/41	49/15	[illegible]	[illegible]	CVH	RVH
6	10	111/[illegible]	113/49	100/30	56/13	9	[illegible]	LVH	LVH
7	10	105/30	118/70	74/25	35/5	18	4.3	CVH	N
8	9	11[illegible]/34	116/83	61/—	[illegible]/5	18	7	CVH	N
9	9	105/40	110/56	60/[illegible]	40/13	6	4	CVH	N
10	9	114/21	135/100	68/40	57/14	4	[illegible]	CVH	N
11	8	105/[illegible]	100/50	95/35	60/40	[illegible]	2.2	RVH	N
12	8	108/41	135/85	65/37	34/16	84	[illegible]	CVH	LVH
13	7	16[illegible]/38	110/[illegible]0	110/37	[illegible]0/30	12	2	CVH	RVH
14	6	114/25	11[illegible]/60	85/85	38/10	4	4	CVH	N
15	6	150/80	120/84	60/40	48/8	4	—	CVH	N
16	5	98/[illegible]4	108/68	68/38	33/5	36	—	CVH	N
17	4	114/30	1[illegible]0/100	[illegible]/50	23/8	[illegible]	3.1	LVH	N
18	4	120/40	114/64	55/22	38/7	12	—	LVH	N
19	3	120/30	105/60	73/11	[illegible]0/7	4	—	CVH	N
20	2	100/40	108/78	70/40	[illegible]/8	12	3.8	CVH	N
21		150/30	100/[illegible]0	118/32	35/8	4	3.9	CVH	N
22	2	130/20	94/60	0/30	2[illegible]/8	[illegible]	—	LVH	N
23	11/12	115/25	108/[illegible]0	70/12	[illegible]	48	2.8	CVH	N
24	10/12	120/23	110/[illegible]5	75/17	1/8	18	—	CVH	N

AVDs = systemic arterio-venous O difference.
AVDp = pulmonary arterio-venous O difference.
N normal electrocardiogram.
CVH = combined ventricular hypertrophy
LVH = left ventricular hypertrophy
RVH = right ventricular hypertrophy

TABLE [illegible]. *The average ratios between the mean systemic and pulmonary arterial pressures and the systemic and pulmonary arterio-venous O differences in different age groups*

Age at operation	Number of children	Mean PAP/mean SAP preop.	Mean PAP/mean SAP postop.	Average AVDs/AVDp
Less than 7 years	11	0.[illegible]	0.19	3.7
7–14 years	13	0.53	0.39	3.0

Mean PAP = mean pulmonary arterial pressure.
Mean SAP = mean systemic arterial pressure
AVDs = systemic arterio-venous O difference
AVDp = [illegible]

Table 3 *Postoperative changes in the average height and duration of the P wave in leads II and V_1*

	P II		P V	
	Average duration in sec.	Average height in mm.	Average duration in sec.	Average height in mm.
Preop.	0.078	2.07	0.065	_45
Postop.	0.070	1.36	0.056	1.69

were not entered at the preoperative catheterization. In these cases nearly arterialized blood was obtained from the main pulmonary artery As a complete admixture of the blood shunted through the ductus and the mixed venous blood may not occur in the main pulmonary artery no calculation of the ratio between the systemic and pulmonary arteriovenous O difference was made in these cases.

Four children were catheterized twice and one child three times after the operation. No significant changes occurred after the first postoperative year (Fig. 1)

Electrocardiography

A normal sinus rhythm was recorded in all cases.

In 18 cases signs of combined ventricular hypertrophy were present before operation Fourteen children in this group had normal tracings after surgery In three cases mild right ventricular hypertrophy persisted after operation (cases 4 5 and 13) These children had preoperative pulmonary arterial pressures ranging from 77 to 120 mm Hg and the shunt was judged to be moderate or small. In one case (case 1) mild left ventricular hypertrophy was present after operation, this was the patient with a mild aortic stenosis.

Two children (cases 2 and 11) had isolated right ventricular hypertrophy preoperatively Both patients had a pulmonary arterial systolic pressure al most equal to the systemic pressure and a small shunt. In both cases the pulmonary arterial pressure decreased only slightly after the operation. The younger of these children had normal electrocardiographic tracings postoperatively the older still showed mild right ventricular hypertrophy at the last examination.

Four children (cases 6, 17 18 22) showed electrocardiographic evidence of isolated left ventricular hypertrophy Three of them were young children with a large shunt Their pulmonary arterial pressures ranged from 55 to 73 mm Hg The electrocardiographic and hemodynamic findings became normal in all these cases after surgery The fourth patient a 10 year old boy showed mild left ventricular hypertrophy at the last examination The follow up time in this case was 9 months.

The decreased load on the ventricles was in all cases reflected by a decrease in the amplitude of the R and S waves in leads V_1 and V The R/S ratio showed a

TABLE 4. *Postoperative changes in the T waves in lead I III and AVL.*

	TI	TIII	T AVL		
	Average height in mm		Positive	Negative	Iso-lectric
Preop.	1.09	..63	1	22	1
Postop.	2.17	0.83	17	4	3

postoperative decrease in lead V_1 in 16 cases and in lead V in 13 cases.

Electrocardiographic signs of combined atrial hypertrophy were usually seen before surgery Postoperatively a decrease in the height of the P wave in lead II was seen in 13 cases, the duration of P_{II} decreased in as many cases. The average value for the height of the P wave in leads II and V_1 decreased postoperatively (Table 3) A smaller decrease occurred in the average values for the duration of the P wave in the same leads.

The T waves tended to be low in lead I and high in lead III before operation. The postoperative changes in the average amplitudes of these waves are shown in Table 4. A negative T wave in lead AVL occurred in 22 cases before operation and in 4 cases after operation.

Discussion

In cases of a patent ductus arteriosus associated with pulmonary hypertension one has to weigh the operative risk against the expected benefit for th patient. It is generally agreed, that operation is usually indicated provided that the shunt is from left to right or that the aortic pressure rises after a test clamping of the ductus [18 19 23 25]. After the operation the progression of the pulmonary vascular changes is probably prevented and the risk of bacterial endocarditis is eliminated.

The effect of the operation on the pulmonary hypertension varies, but in the majority of cases a decrease of the pulmonary arterial pressure occurs [1 3 4 10 12, 19 20 21 ..4, 25]. The effect of the operation on the pulmonary vascular resistance is less clear The calculation of the preoperative pulmonary vascular resistance is subject to considerable inaccuracy especially in the cases where the difference in the oxygen values in the aorta and in the pulmonary artery is very narrow [3 9, 14 20] Usually the drop of the pulmonary arterial pressure parallels the drop in the pulmonary blood flow but there are reports, in which a decrease of the pulmonary vascular resistance has occurred after closure of the ductus. Cournand *et al.* [8] catheterized a three year old girl 5 weeks after operation for a patent ductus and found an elevated pulmonary arterial pressure and pulmonary vascular resistance. At a second catheterization 4 months later these values were within normal limits. Other investigators have made similar observations in solitary cases [.. 4 17 21]. Braunwald *et al.* [3] observed that of 6 patients with large left to right shunts on the pulmonary artery level and increased ratio of the pulmonary

vascular to the systemic vascular resistance, 5 had normal values after operation. They conclude that there is better chance of postoperative regression of the elevated pulmonary vascular resistance in patients with extracardiac than in those with an intracardiac shunt.

On the other hand there is a group of patients with a patent ductus and pulmonary hypertension whose pulmonary vascular disease seems to be primary in nature [23]. Rudolph *et al.* [20] reported an infant who showed rapid progression of the pulmonary hypertension and pulmonary vascular resistance after operative closure of the ductus. In the series described by Reid *et al.* [19] one patient showed two years postoperatively a pulmonary pressure which equalled the systemic pressure. Other authors have described similar cases [4 17 19 25].

In the present series all patients showed a decrease in the pulmonary arterial systolic pressure. The pressure became normal or almost normal in cases with a big shunt. Most children below 6 years of age had normal hemodynamic findings after operation. This age group had also on an average larger shunts than the older children. Braunwald *et al.* [3] found in their series of children with a left to right shunt and pulmonary hypertension no evidence that the age of the patient should influence the effect of the operation on the pulmonary hypertension.

It seems possible to some extent to predict from the electrocardiogram the effect of the operation on the pulmonary hypertension. In the cases where marked right ventricular hypertrophy was present before operation only slight to moderate decrease of the pulmonary arterial pressure occurred. Persisting right ventricular hypertrophy after operation was usually a sign of persisting pulmonary hypertension. A normal electrocardiogram at this stage did, however not exclude a moderate pulmonary hypertension.

The diminished load on the right atrium was reflected in the change in the height of the P wave in leads II and V [22]. The signs of left atrial enlargement, broad P waves in lead II and terminal negativity in the P waves in lead V_1 [22] disappeared after surgery.

The postoperative change in the T waves was most consistent in lead AVL in which negative T waves were seen before surgery in 22 cases and after surgery in 4 cases. A negative T wave in lead AVL may occur in normal vertical hearts, but is rarely seen in normal children [23]. It is common in left ventricular hypertrophy [11 22]. Landtman [15] observed a negative T wave in 20 of 3 children with an uncomplicated ductus arteriosus and in 2 of 46 children with pure pulmonary stenosis. In our series the only child who had a positive T wave in lead AVL before and after the operation, had mild aortic stenosis as a complicating lesion.

The postoperative increase in the height of T_I and decrease in the height of T_{III} can probably be explained by the disappearance of the left ventricular hypertrophy [6, 22].

It is interesting that neither progression nor further decline of the pulmonary arterial pressure occurred in the few patients who were catheterized more than once after the operation. More serial studies in these patients will be needed before their longterm prognosis will be known.

Heath *et al* [13] have related the reversibility of the pulmonary hypertension in congenital heart disease to structural changes in the pulmonary vessels and have graded these according to their severity They found that a lung biopsy is of value in assessing the severity of the pulmonary vascular disease and the prognosis of the patient. In our series no correlation could be found between the degree of the pulmonary vascular changes and the severity of the clinical findings. On the other hand, Landtman & Hjelt [16] observed extensive histological changes in the lung biopsies from patients with a patent ductus arteriosus but no pulmonary hypertension. They suggested that the development of the vascular changes could be governed by individual variations in the resistance and adaptability of the pulmonary vascular bed to the increased blood flow

Summary

Pre- and postoperative hemodynamic and electrocardiographic studies were carried out in 24 children with patent ductus arteriosus and pulmonary hypertension. The age of the patients ranged from 10 months to 14 years. All patients were catheterized before and at least once after the operation. In all cases a left to right shunt was present Prior to operation the pulmonary arterial pressure was 50 mm Hg or more. The follow up time ranged from 6 months to 4½ years

Clinical improvement and a decrease of the pulmonary arterial pressure occurred in all cases after operation. The postoperative systolic pressure in pulmonary artery was 40 mm Hg or less in 14 children, all these children had had large shunts and 10 of them were less than 7 years of age In the 4 cases in which more than one post operative catheterization was performed, no significant hemodynamic changes were seen after the first postoperative year

Before operation 18 patients had electrocardiographic signs of combined ventricular hypertrophy Following surgery the tracings became normal in most cases. Isolated right ventricular hypertrophy in the preoperative or persisting right ventricular hypertrophy in the postoperative tracings were unfavourable signs and occurred in cases, in which only a small lowering of the pulmonary arterial pressure occurred.

References

1. Adams, P., Adams, F H., Varco, R. L., Dammann J F and Muller, W H.: Diagnosis and treatment of patent ductus arteriosus in infancy *Pediatrics* *12*, 664, 1953.
2. Anderson, L M. and Collis, H. M. T.: Patent ductus arteriosus with pulmonary hypertension. *Thorax*, *10* 338, 1955.
3. Braunwald, N S., Braunwald W and Morrow A. G The effects of surgical abolition of left-to-right shunts on the pulmonary vascular dynamics of patients with pulmonary hypertension. *Circulation*, *26* 1270, 1962.
4. Burchell, H. B Regression of pulmonary hypertension after cure of intracardiac defects. In *Pulmonary Circulation* Grune & Stratton New York, 1959, p. 248.
5. — Variations in the clinical and pathologic picture of patent ductus arteriosus. *Med Clin N Amer* *32* 911 1958
6. Butsenko, K. P Electrocardiogram in patent ductus arteriosus and its changes after closure of the duct. *Exp Khir Anest*, *9* 11, 1964
7. Cabrera, E. and Monroy J R.: Systolic and diastolic loading of the heart. Physiological and clinical data. *Amer Heart J* *43* 661 1952.
8. Cournand H., Baldwin J S. and Him

in … A.: In *Cardiac catheterization in con… heart disease*, The Common Wealth Fund, New York, 1949 p. 89.

9 DEXTER, L.: Cardiac catheterization in diagnosis of congenital heart disease. *Minnesota Med*, 37 118, 1954 quoted by Keith *et al.*

10 EKSTRÖM, G The surgical treatment of patent ductus arteriosus. A clinical study of 290 cases. *Acta Chir Scand* Suppl. *169* 119 1952.

11 FRIEDBERG, C. K.: *Diseases of the heart* W B Saunders Company Philadelphia and London, 1956, 2nd edition.

12. HARRIS P Patent ductus arteriosus with pulmonary hypertension. *Brit Heart J* *17* 85, 1955.

13. HEATH D HELMHOLZ, F JR., BURCHELL, H. B., DU SHANE, J W KIRKLIN J W and EDWARDS, J E.: Relation between structural changes in the small pulmonary arteries and the immediate reversibility of pulmonary hypertension following closure of ventricular and atrial septal defects. *Circulation* *18*, 1167 1958.

14. KEITH, J D ROWE, R. D and VLAD P *Heart disease in infancy and childhood.* The MacMillan Company New York, 1958.

15. LANDTMAN, B Postoperative changes in the electrocardiogram in congenital heart disease. II. Coarctation of the aorta and patent ductus arteriosus. *Circulation*, *10* 871 1954.

16. LANDTMAN B. and HJELT, L. Pulmonary vascular changes in patent ductus arteriosus. *Ann Paediat Fenn*, *3* 37 1957

17 LARON R. L.: Pulmonary hypertension in patent ductus arteriosus. In *Pulmonary Circulation*, Grune & Stratton, New York, 1959 p. 216.

18. NADAS, A. S.: *Pediatric cardiology* W B. Saunders Company Philadelphia and London, 1963.

19. REID, J M., STEVENSON, J G COLEMAN E. N BARCLAY R. S., WELSH, T M., FYFE, W M. and INALL, J A.: Moderate to severe pulmonary hypertension accompanying patent ductus arteriosus. *Brit Heart J* *26* 600 1964.

20. RUDOLPH A. M., MAYER, F E., NADAS, A. S. and GROSS, R. E.: Patent ductus arteriosus. A clinical and hemodynamic study of 23 patients in the first year of life. *Pediatrics* *22* 892, 1958.

21 SILVER, A. W ELLIS, F H., KIRKLIN, J W and WOOD, E. H Regression of pulmonary hypertension after closure of patent ductus arteriosus. *Proc Staff Meet Mayo Clin*, *29* 293 1954

22. SODI PALLARES, D and CALDER, R. M.: *New bases of electrocardiography*. Henry Kimpton, London, 1956, 2nd edition.

23. TAUSSIG, H.: *Congenital malformations of the heart.* The Common Wealth Fund, Cambridge 1960.

24. TERKELSEN FR., GAMMELGAARD A. and BONNEVIE L.: Kongenitte Hjertelidelser hos spaedbørn. *Ugesk Laeg*, *126* 1237 1964.

25. TOUJI, H., SHAPIRO, M., MADDISON O DUNNE, E., DYKSTRA, P and KAY J H.: Surgical treatment of high pressure patent ductus arteriosus. *Circulation*, *27* 632, 1963.

26. WOOD, P *Diseases of heart and circulation*, Eyre & Spottiswoode, London, 1956.

27 — Pulmonary hypertension with special reference to the vasoconstrictive factor *Brit Heart J* *20* 537 1958.

28. ZIEGLER, R. F.: *Electrocardiographic studies in normal infants and children.* C. Thomas, Springfield, Ill., 1951.

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Serum Lipids in Premature Infants on Vegetable Fat Diet

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It is well known that serum cholesterol in adults is lowered when animal fat in the diet is replaced with vegetable fat. This effect is ascribed to the high content of unsaturated fatty acids in vegetable fat. These findings are of great importance in the study of the pathogenesis of atherosclerosis.

In pediatrics such problems have so far not been considered of interest, since atherosclerosis is very uncommon in children, although not quite unknown. Thus, Kube & Saolowjew [0] have described fatty infiltration of the intima of the aorta in 1–6-month-old infants, and similar changes have been found in children over 3 years of age by Holman *et al* [7]. Rare cases of calcification of the coronary arteries have been reported in early childhood [16]

As only very few reports on the serum lipids of premature infants on vegetable fat diet have been published [5 1–, 10], we have considered it of interest to present this study

Material and Methods

We have used a dried milk preparation, Olac, consisting of cow milk where the fat has been replaced with vegetable fat so that the content of unsaturated fatty acids has become similar to that of human milk. Besides, a large part of the casein has been removed, and iron and vitamins have been added. The formula ready for use contains 3.3% of fat, 0.6 % of casein, 0.6% of albumin + globulin and 7.–% of lactose The lipid fractions are listed in Table 1

The composition of this formula is very similar to that of Milkotal[b] employed by Holmberg *et al.* [8] in the feeding of full term infants.

In the present investigation three groups of premature infants were studied:

Group I comprised 19 premature infants who started on human milk, when they had attained a weight of 2000–2500 g (average 2160 g) the diet was changed to Olac The age of the infants at this time differed rather much (10–56 days) owing to differences in birth weight After a period of 2–5 weeks (average 22 days) the diet was again changed, this time to a 50% cow milk formula (as commonly used in this country). Blood samples for lipid analyses were drawn *just before* the beginning of the Olac period, two weeks later and in some cases again two weeks later

Group II was a control series of 12 infants who also started on human milk, but they continued with this until their weight was 2 00–2900 g Thereafter they received 50% cow milk formula. Blood samples were drawn with the same intervals as in group I.

Group III was an additional control series of 10 infants whose serum lipids were

Kindly supplied by Leo Pharmaceutical Products, Ltd., Denmark.

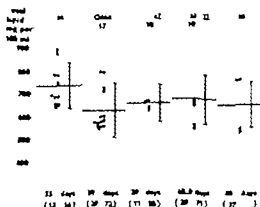


Fig. 1. Total lipids in serum. Columns 1 and 2 represent infants of group I; columns 3 and 4 group ..., and column 5 group 3. HM human milk; HMCI human milk, control I; HMCII human milk, control II; CM cow milk. The number in each column is the number of infants examined.

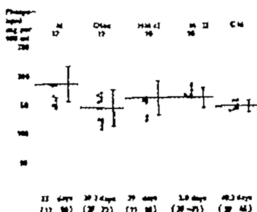


Fig. 2. Phospholipids in serum. Abbreviations as in Fig. 1.

determined only on 50% cow milk formula, after this had been given for at least one week.

Analytical methods. Total serum lipids were determined with the method of Schoenheimer & Sperry [15], cholesterol according to G. C. Brun [1] and phospholipids with the method of G. Brun [2]. The determinations were carried out in the Medical Laboratory, Gutenberghus, Copenhagen.

Results

The total lipids in serum (Fig. 1) were a little lower when the infants received Olac than on human milk, but the difference was not statistically significant. There were no differences between the results obtained on 50% cow's milk formula and on Olac.

With regard to the phospholipids, quite similar results were obtained (Fig. 2).

Serum cholesterol (Fig. 3) was on an average 101.. mg per 100 ml (S.D. = 18.4 mg per 100 ml) when Olac was given, and 120.3 (S.D. = 4.1) on human milk. This difference was statistically significant ($P < 0.0..$). Thus, a fall in cholesterol was seen when the diet was changed from human milk to Olac, but this was not due to the infants growing older, since only a very small, not significant, fall was seen in the control group (group II) that in a similar period of life remained on human milk. On 50% cow's milk formula, the cholesterol level was 1?1.4 mg per 100 ml (S.D. = 13.8), which is very similar to that of the infants on human milk, the two groups being comparable with regard to age and weight.

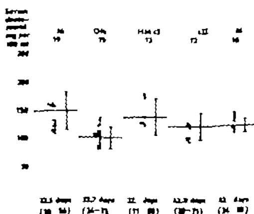


Fig. 3. Serum cholesterol. Abbreviations as in Fig. 1.

All the infants tolerated Olac well and thrived as well as the controls. The study however was not planned to evaluate the weight gain.

Discussion

Investigations on the dependence of the serum lipids on the content of animal or vegetable fat in the food have been done not only in adults, but also in full term infants [4, 10 1_, 13, 17 18 20 *1] In contrast to this only a few reports dealing with premature infants have been published [5 1* 19] György *et al.* [5] examined a series of 327 premature infants who received a cow's milk formula con taining _7% of protein and 2.8% of butter fat, and measured the levels of serum cholesterol and total lipoprotein. On another formula, prepared from cow s milk and containing 1.5% of protein and 3.3% of vegetable fat with a high content of unsaturated fatty acids, considerable lower levels of cholesterol and lipoprotein (particularly β-lipoprotein) were found. These results are in accordance with those obtained in full-term infants.

On the other hand, Verga & Potot sching [19] found no changes in the serum cholesterol of premature infants who received a formula where 20% of the calories were given as vegetable fat with 10% of linoleic acid.

In accordance with Gyorgy [5] and the previous authors who have examined full term infants we have found that serum cholesterol is significantly lower on a vegetable fat diet than on human milk. This is difficult to understand when we consider the similarity of the content of unsaturated fatty acids in the vegetable fat diet employed and in human milk.

TABLE 1 *Comp nt fatty acids in human milk, Olac and cow s m lk in per cent of total fat*

	Human milk	Olac[b]	Cow milk
Butyric acid	0	0	2.5–4
Caproic acid	0	0	2–3
Caprylic acid	0 5–1	1.0	1 2
Caprinic acid	0 3–1	0.9	3–5
Lauric acid	5–7	6	4–5
Myristic acid	5–14	4	5–14
Palmitic acid	21–25	27	23–35
Stearic acid	7–14	11	10–15
Arachidic acid	1–	0.3	0–
Myristoleic acid	0–0 5	0.2	0.5–_5
Palmitoleic acid	2.5–5	1 4	1 3
Oleic acid	30–40	37	20–30
Linoleic acid	5.5–10.5	10	0–1
Other octadecadienoic acids	0	0	0.5–
Linolenic acid	0.5–1.2	0.9	0–1
Stearidonic acid	0 1	0	0
Arachidonic acid	0.1–0.4	0.3	0
Total polyunsaturated acids (approx.)	9	11	2
Total unsaturated acids (pprox.)	48	50	31
Total fat (g per 100 ml)	3.5	3 3	3.5

After Holmberg *et al* [8].
According t the manufacturer

We have also found that the serum cholesterol is practically identical on human milk and on a 50 _ cow s milk formula, in spite of the fact that the latter contains less than one-third of the amount of unsaturated fatty acids present in the former (Table 1) Furthermore, only part of the polyunsaturated fatty acids in cow's milk is linoleic acid, the remainder being an isomeric diene which probably is not essential for man [8]. Other authors have made similar observations.

The above-mentioned unexplainable facts suggest that other factors besides the total amount of unsaturated fatty

acids must play a role. It is not evident from our figures that any specific fraction of the unsaturated fatty acids is of importance in this respect, in particular the total amount of polyunsaturated fatty acids apparently is not the decisive factor.

Rafstedt [14] found, in accordance with other authors [4, 10, 12, 17, 18, 21], a gradual increase of the serum lipids in the first days and weeks after birth. He examined both full-term and premature infants and found that the increase was a little slower in the prematures. Serum cholesterol increased from 67 mg per 100 ml at birth to 117 mg per 100 ml 3–26 days later (average values).

Our findings regarding the dependence of serum cholesterol on age differ from this, as we found a slight, not significant fall with age in the control series (group II) that remained on human milk. However, our infants were a little older (age ~7 weeks) than those mentioned by Rafstedt; none of ours were under 10 days of age.

In adult age it is believed to be more healthy to have a low serum cholesterol, but similar opinions are difficult to hold with regard to infants and children. One might reasonably suppose that the natural food, human milk, causes the most "normal" level of serum cholesterol. We do not know whether the low serum cholesterol obtained through a vegetable fat diet is beneficial or harmful. For the present, it does not seem justified to start the prevention of atherosclerosis in early infancy.

Severe deficiency of unsaturated fatty acids, particularly linoleic acid, gives rise to various symptoms, especially in the skin which becomes dry, scaly and thickened [6]. Apparently the moderately low content of unsaturated fatty acids in cow's milk formulas does not cause any symptoms. It is, however, possible that the more delicate skin of breast-fed infants may be ascribed to the higher content of unsaturated fatty acids in human milk, but this has not been further investigated. It should be mentioned that a similar delicate skin has been observed in infants after feeding with Pelargon®, a cow's milk preparation whose butter fat has not been altered [3].

Summary

Serum lipids were studied in premature infants on different diets: human milk, an ordinary 50 % cow's milk formula and a cow's milk preparation (Olac) where the butter fat had been replaced with vegetable fat, so that the content of unsaturated fatty acids was practically similar to that of human milk.

The total lipids and phospholipids showed no significant differences on these diets. Serum cholesterol was significantly lower on feeding with Olac than on human milk, but no differences with regard to the cholesterol were seen between infants on human milk and infants on 50 % cow's milk formula. These findings are in accordance with previous investigations.

As the amount of unsaturated fatty acids is practically identical in human milk and in Olac, we cannot explain why serum cholesterol is lower when the latter is given. Nor can it be explained why serum cholesterol is similar on human milk and on 50 % cow's milk formula, the content

of unsaturated fatty acids in fact being more than three times as high in the former as in the latter.

Apparently the total amount of polyunsaturated fatty acids is not the factor responsible for these results. Our figures do not permit the conclusion that any single fraction of the lipids is the factor in question. This problem needs further investigation.

References

1. Bruun, Georg C. En mikrometode til bestemmelse af frit og esterbundet kolesterin i serum eller blod. *Hospitalstidende, 78*: 688, 1935.
2. Bruun, Gudrun: Lipoidfosforbestemmelse i serum. *Bibl Laeg, 131*: 203, 1939.
3. Dyggve, H. Ernæring af spædbørn med syrnet sødmælkspulver (Pelargon®). *Ugeskr Laeg, 121*: 1011, 1959.
4. Goalwin, A. and Pomeranze, J. Serum cholesterol studies in infants. (A comparison of infants fed breast milk, evaporated milk and corn oil formulas.) *Arch Pediat, 79*: 56, 1962.
5. György, P., Rose, C. S. and Chu, E. H.: Serum cholesterol and lipoproteins in premature infants. *Amer J Dis Child, 104*: 165, 1962.
6. Hansen, A. E., Stewart, R. A., Hughes, G. and Söderhjelm, L. The relation of linoleic acid to infant feeding. *Acta Paediat Scand*, Suppl. *137, 51*: 1–41, 1962.
7. Holman, R. L., McGill, H. C. Jr., Strong, J. P. and Geer, J. C.: Arteriosclerosis—the lesion. *Amer J Clin Nutr, 8*: 85, 1960.
8. Holmberg, J., Jacobson, E. and Malmberg, M.: Synpunkter på sammansättningen av mjölk, avsedd för spädbarn. *Svensk Läkartidn, 51*, 1100, 1954.
9. Kuhn, T. and Sokolowjew, A. Über die Lipoidablagerung in der Aorta von Kindern im frühen Säuglingsalter. *Frankfurt Z Path, 40*: 307, 1930.
10. Lindquist, B. and Malmcrona, R. Dietary fat in relation to serum lipids in the normal infant. *Amer J Dis Child, 99*: 39, 1960.
11. Omans, W. B., Leuterer, W. and György, P. Feeding value of soy milks for premature infants. *J Pediat, 62*: 98, 1963.
12. Pomeranze, J., Goalwin, A. and Slobody, L. B.: The effect of corn oil-evaporated milk mixture on serum cholesterol levels in infancy. *Amer J Dis Child, 95*: 622, 1958.
13. — Infant-feeding practices and blood cholesterol levels. *Amer J Clin Nutr, 8*: 340, 1960.
14. Rafstedt, S. Studies on serum lipids and lipoproteins in infancy and childhood. *Acta Paediat Scand* Suppl. *102, 44*: 1–109, 1955.
15. Schoenheimer, R. and Sperry, W. M. A micromethod for the determination of free and combined cholesterol. *J Biol Chem, 106*: 745, 1934.
16. Stryker, W. A.: Arterial calcification in infancy with special reference to the coronary arteries. *Amer J Path, 22*: 1007, 1946.
17. Sweeney, M. J., Etteldorf, J. N., Dobbins, W. T., Somervill, B., Fischer, R. and Ferrell, C. Dietary fat and concentrations of lipid in the serum during the first six to eight weeks of life. *Pediatrics, 27*: 63, 1961.
18. Sweeney, M. J., Etteldorf, J. N., Obran [?], P. M. and Fischer, R.: Effect of diet during the first six to eight weeks of life on total concentrations of serum proteins and on electrophoretic patterns of serum protein and lipoprotein. *Pediatrics, 29*: 82, 1962.
19. Verga, A. and Pototschnig, C. Dietary fats in infant nutrition. Clinical investigation with dried milk of modified fatty composition in the premature infant. *Minerva Pediat, 15*: 377, 1963.
20. Woodruff, C. and Coniglio, J. G. Studies on lipids in infancy: Maternal-infant differences and the effects of human and cow milk feeding. *Amer J Dis Child, 98*: 638, 1959.
21. — Studies on lipids in infancy: Comparison of human and cow milk feeding. *Amer J Dis Child, 100*: 644, 1960.

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Malabsorption of Vitamin B_{12}

Report of a Case in a 1 Year-old Boy Including Studies of the Absorption of B_{12}

by ERIK HIPPE

In 1960 Imerslund [12] and Gräsbeck *et al.* [8] independently described a characteristic syndrome occurring in children and comprising chronic megaloblastic anaemia and proteinuria. A large proportion of Imerslund's patients had associated anomalies of the urinary tract. The gastric juice contained free hydrochloric acid and intrinsic factor.

Imerslund's material comprised ten patients from six different families in south-east Norway while Gräsbeck *et al.* reported two cases from Finland. In addition, in 1952 Najman & Brausil [18] reported from Yugoslavia an 18-month-old girl with megaloblastic anaemia who also had proteinuria. Her gastric juice contained free hydrochloric acid and intrinsic factor. On oral liver administration the blood picture became normal. Other cases were described by Collo *et al.* [4], Lamy *et al.* [14] in 1961, Sievers [21] in 1962 and Spurling *et al.* [22] in 1964.

Thus far only 21 cases have been reported, but even so there has been considerable interest in this disorder since its investigation has helped to shed new light on the nature of the intestinal absorption of vitamin B_{12}.

In all probability the defect is congenital, since the time of onset of the symptoms corresponds to the expected period of depletion of the child's vitamin B_{12} depots, which are stored prenatally by transfer from the maternal blood. The reason why the megaloblastic anaemia is associated with proteinuria and in many instances with renal anomalies in these patients is not known. No doubt a genetic factor [1?] is operational, and presumably the characteristic syndrome may be caused by an incidental and closely related combination of genes. However, the series of cases hitherto published are too limited to allow a more detailed delineation of the genetic conditions.

The prognosis is generally good, provided that adequate parenteral vitamin B_{12} therapy is instituted, which will, presumably have to be continued for the rest of life.

In 1963, Imerslund & Bjørnstad [13] reported on a follow up study of the ten patients previously described. At that time the patients were between 7 and 24 years of age. Growth and development were found to be normal. In all cases the proteinuria persisted. Renal function was normal. The gastric juice contained free hydrochloric acid and intrinsic factor. Following cessation of therapy the condition in all cases recurred in about 15 months.

In an attempt to reveal the cause of malabsorption of vitamin B_{12}, Collo *et al.*

[4] administered radioactively labelled vitamin B_{12} orally and determined the urinary excretion (Schilling test) The excretion was found to be negligible. When jejunal juice was administered together with the labelled vitamin B_{12}, normal intestinal absorption of vitamin B_{12} was obtained. The authors suggested that normal intestinal juice contains a factor required for the absorption of vitamin B_{12} and that the failure to absorb in this disease is due to the absence of this factor Björnstad & Imerslund [1] and Spurling et al. [22] however were not able to confirme these findings.

In the following we wish to report a year old boy who both clinically and with regard to pathogenesis seems to belong to this *category of disease. The absorption* of vitamin B_{12}, and the failure to absorb in this disorder is discussed.

Case Report

A 16-month-old boy was first admitted to the hospital in February 1963 because of anaemia diagnosed at routine examination at the age of 15 months.

H is an only child. His father brother died at three years of age after hemmephrectomy because of double kidney and ureter complicated by hydronephrosis and pyelonephritis. In addition, a sister of his paternal grandfather had unilateral double kidney and ureter

Delivery was normal. Birthweight 3100 g, crown-to-heel length 47 cm. The child was breast-fed till he was one month old and was then given increasing concentrations of cow's milk in sugar water At 3 months mashed potatoes and vegetables were added t the diet. From the age of 6 months he lived mainly on porridge, he was given ½ l of milk daily and small quantities of vegetables and meat. From the ge of 3 weeks and to the age of 15 months an additional supply of vitamins A, D and C was given.

On routine m dical examination at the age of 5 weeks the haemoglobin was 14.8 g/100 ml (100%) no albumin and no sugar in the urine.

Development had been completely normal. H had frequent ttacks of upper respiratory tract infection and alternating episodes of constipation and diarrhoea. Shortly before admisson he had dermatitis around the anus.

The anaemia was revealed at a routine examination when the child was 15 months old. The findings were then Hb. 9.6 g/100 ml (65%), urine: no albumin.

The patient was then given an oral iron preparation (Glycifer®), but in spite of this therapy the fall in haemoglobin alues continued and he was, therefore, admitted to hospital 16 months old for further examination.

Physical examination revealed a boy whose general appearance and development was normal for his age. His nutritional stat was normal (weight 10,430 g, crown-to-heel length 80 cm). H appeared somewhat tired and apathetic, very pale with numerous haemorrhages into the skin of the trunk and the extremities. Abdomen was rather large but no enlargement of liver or pleen was found. N signs of infection or palpable lymph nodes were revealed.

Laboratory data prior t institution f specific therapy- Haemoglobin 6.9 g 100 ml (47%) MCV 1?6 μ^3 (normal range 86–96 μ^3). MCHC: 31.2 g/100 ml Mean erythrocyte diameter: 2–9 μ. Anisocytosis and poikilocytosis. Furthermore peripheral blood smear showed: 2% erythroblasts and 2% megaloblasts, 92 % lymphocytes and 4% granulocytes. Reticulocytes: 0.4%. Serum bilirubin 1.3 mg/100 ml Erthrocyte sedimentation rate: 8 mm hour Whit cell count 4300 mm Platelets 75,000 mm Serum folic acid: normal. Vitamin B_{12} in serum. 30 μg ml (normal range 150– 50 μg ml) Microscopical xamination of tibial bone marrow revealed megaloblastic anaemia. Serum protein electrophoresis normal. Gastric juice contained free hydrochloric acid. 63 mEq l, and intrinsic factor Serum

contained no antibodies against intrinsic factor. Stool specimens contained no parasitic ova. Xylose tolerance test normal. Gastrointestinal series with contrast revealed normal findings.

As mentioned above the urine had been examined since the age of five weeks and till one month before admission, and protein had never been present. From the age of 16 months there was a persistent proteinuria, ranging from 0.01 to 0.22%. Electrophoretic examination of urine: 45% albumin, 1% α_1, 11% α_2, 16% β, and 17% γ-globulins (i.e. a relatively high content of γ-globulin). Chromatographic determination of amino acids revealed normal findings. Serum creatinine: 0.2 mg/100 ml. Urography normal.

In connection with the Schilling test (cf. below) 0.5 mg of Cyanocobalamin was injected intramuscularly. As early as 4 days thereafter a marked increase in the reticulocyte count was observed, reaching a *maximum (1.4%) on the th day (Fig. 1)*. The haemoglobin value remained fairly constant during the first week (10.2 g/100 ml) and rose after that time to reach normal values in the course of one month (14.0 g/100 ml).

Serum iron was not determined until one week after the injection of vitamin B_{12}. The value was then 26 µg/100 ml (normal range 45–165 µg/ml). Serum transferrin: 74 µg/100 ml (normal range 250–400 g/100 ml). Two months later: serum iron: 45 µg/100 ml and serum transferrin: 612 µg/100 ml.

The Schilling test (Table 1) showed virtually no intestinal absorption of vitamin B_{12}, both with and without the addition of intrinsic factor.

After the child had been given aspirated duodenal juice and jejunal juice, respectively, the Schilling test showed no increase in the intestinal absorption of vitamin B_{12}. The intestinal juice was obtained from two patients during two otherwise uncomplicated gall bladder operations by aspiration through Miller-Abbott tubes, the first on a level with the ascending part of the duodenum and the other about 60 cm distal to the pylorus. The negative Schilling test might be caused by inhibition of the intestinal secretion owing to anaesthesia or surgery.

A repeat Schilling test was now done in our patient with administration of 100 ml of intestinal juice obtained from two volunteers by aspiration of 50 ml from each through inlying Miller-Abbott tubes over a period of about 12 hours and from a site 1–1.5 m down the small intestine. This Schilling test revealed heavily increased intestinal absorption of vitamin B_{12} (Table 1).

During a subsequent admission we administered 20 ml of Dustivit® in which vitamin B_{12} is bound to a peptide, over a period of six days. The daily dose corresponded to about 20 µg of B_{12}, or about three times the average dietary content. This concentration is not so high that a possible absorption could be explained exclusively as a physical phenomenon. Then we followed up the reticulocyte values (Fig. 2) and as early as after three days an increase was observed, reaching a maximum at the seventh day.

True enough the reticulocytes did not exceed two per cent, but this may be due to the fact that at that time the patient was not anaemic. By contrast, we checked the serum-B_{12} value and found an increase from 176 to 750 pico-g/ml.

At present our patient has been followed up for one year. The haemoglobin value has remained normal on parenteral vitamin B_{12} therapy; there is persistent proteinuria, the renal function is normal and development has been normal.

In summary this patient suffers from a megaloblastic anaemia caused by deficient supply of vitamin B_{12} to the bone marrow as demonstrated by the low serum B_{12} value and the pronounced and instantaneous response to parenteral administration of vitamin B_{12} (Fig. 1). The low serum iron values subsequent to commencing therapy must be a result of an increase in the iron requirements of the bone

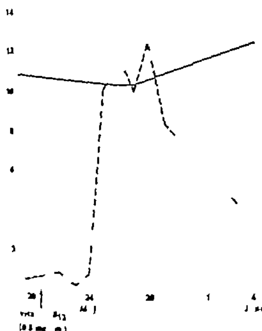


Fig. 1. Hemoglobin and reticulocyte response to the first vitamin B_{12} injection. — Hemoglobin g/100 ml; – – – – reticulocytes %.

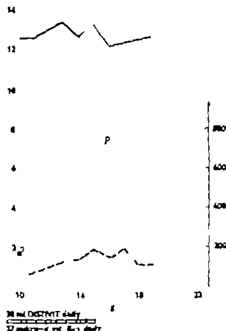


Fig. 2. Orally administered peptide-bound vitamin B_{12} (Dostivit). — Hemoglobin g/100 ml; – – – – reticulocytes % serum vitamin B_{12} level pico-g/ml.

marrow [9] Two months later determinations of serum iron revealed normal values.

The patient had received a normal varied diet, and consequently the dietary intake of vitamin B_{12} must be presumed to have been adequate.

There is normal content of intrinsic factor in the gastric juice, in addition to free hydrochloric acid, and serum does not contain antibodies against intrinsic factor Control measurements of serum B_{12} following parenteral administration of vitamin B_{12} (Fig 2) reveal normal values, and the patient serum must, therefore, be presumed to have a normal protein binding capacity for vitamin B_{12}.

The Schilling test in connection with a normal xylose tolerance test shows that the patient suffers from a selective mal absorption of vitamin B_{12}.

Not until normal intestinal juice (Tabl

Table 1 *Data on Schilling test*

Date	Test dose		in urine
21.05.65	$Co^{58}B_{12}$		0 4
27.05.65	$Co^{57}B_{12}$ + IF		0 5
17 06.65	$Co^{58}B_{12}$ + duodenal juice		0 3
29 09.65	$Co^{58}B_{12}$ postoperat	jejunal juice aspirated	0.3
15.10.65	$Co^{58}B_{12}$ + normal	through Miller Abbot tubes	5 3

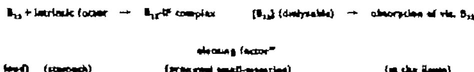


Fig. 3. Intestinal absorption of vitamin B_{12}. (Ref. V. Herbert, B. A. Cooper and W. B. Castle [5, 11].)

1) or a vitamin B_{12} peptide (Fig.) was administered, was intestinal absorption of vitamin B_{12} restored to normal.

Discussion

The intestinal absorption of vitamin B_{12}

In 1959 Booth & Mollin [_] proved that the absorption of vitamin B_{12} occurs in the ileum. This explains the development of megaloblastic anaemia caused by vitamin B_{12} deficiency after extensive resection of the ileum [3, 6], terminal ileitis Whipple's disease and fistulae between the intestines resulting in a by-pass of the distal ileum [7]. By contrast normal absorption of vitamin B_{12} has been found following resection of the jejunum [ ]. Booth & Mollin concluded that the mechanism of absorption of vitamin B_{12} must depend on a specific receptor mechanism in the ileum, and that theoretically it should be possible to reveal a congenital defect in this receptor mechanism which might result in a clinical syndrome similar to pernicious anaemia.

On the basis of the studies hitherto performed it is suggested [5, 7, 10] that the absorption of vitamin B_{12} takes place through the following three stages:

1. Binding of vitamin B_{12} to intrinsic factor.
2. Adsorption of the B_{12}-intrinsic factor complex (B_{12}-IF) to the intestinal mucosa. This process requires Ca.
3. An energy requiring passage of vitamin B_{12} into the intestinal wall.

Cooper & Castle [5] proved that normal rat intestine contains a factor which makes vitamin B_{12} dialysable, a property not possessed by B_{12}-IF. For absorption to occur B_{12} must be capable of being dialysed. They advanced the theory that the B_{12}-IF is split, probably by an enzymatic liberation under the influence of a so-called "releasing factor" (Fig. 3). By experiments with rats, Herbert *et al.* [11] showed that the factor is secreted in the proximal 10 % of the small intestine. This may be difficult to understand when considering that the absorption takes place in the distal parts, viz. the ileum [2], but can be likened to the fact that the intrinsic factor is also secreted proximally to its site of function, thereby allowing the factor some time to exert its action during the process of transport through the intestine. Herbert [10] demonstrated the presence of a similar "releasing factor" in the human small intestine.

Conclusion

In the entity described by Imerslund and Gräsbeck *et al.* a factor which is necessary for the absorption of B_{12} is lacking. However, in 6 of the further 10 described patients (4 not tested) of Bjørnstad & Imerslund [1] and in the * cases of Spurling *et al.* [22] there could not be demon

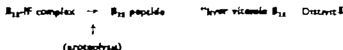


Fig 4. Theory: "releasing factor" = proteolytic enzyme

strated any effect of intestinal juice on the absorption of vitamin B_{12}. The fact that the juice was ineffective in these cases, contrary to that of Colle *et al* [4] and that of our presented case, may be due to secondary changes in the intestinal wall arisen in the course of the patients life, or a different biochemical defect. In the experiments described by Colle *et al.*, and in the present paper (Table 1) normal intestinal juice increased the absorption of vitamin B_{12}, which may be caused of a factor identical with the releasing factor described by Cooper & Castle [5] That lack of a similar factor may occur in adults is shown by the cases described by Resnick *et al.* [20] and Moritt *et al* [17].

If we presume that the factor is a proteolytic enzyme which is capable of splitting the B_{12}-IF complex to form a B_{12} peptide, which is then absorbed, it is probable that B_{12} reaches the liver in this form and is stored most likely as B_{12} peptide (Fig 4)

This is in agreement with Reitzenstein's [19] demonstration of a higher absorption of liver vitamin B_{12} than of crystalline vitamin B_{12} on oral administration, in particular in patients with pernicious anaemia, and it serves to explain why pernicious anaemia can be successfully treated with raw liver

If, in other words, the selective malabsorption of vitamin B_{12} is caused by a defect in the proteolysis of the B_{12}-IF complex and the formation of vitamin B_{12} peptide, the patient should be able to absorb vitamin B_{12} when administered in a peptide-bound form.

On administration of such a vitamin B_{12} peptide (Dastivit®) we found that this theory was substantiated, since an increase in the reticulocyte count and in the serum vitamin B_{12} ensued (Fig)

Furthermore, previous investigations [15 16] have shown that patients suffering from other types of malabsorption of vitamin B_{12} have been able to absorb peptide-bound vitamin B_{12} These findings therefore, support the theory that the final structural composition of vitamin B_{12} at the time of absorption is as a vitamin-peptide complex. The peptide chain is most likely the residue of the intrinsic factor after its separation from B_{12} by releasing factor

Summary

A 1 year-old boy with a characteristic entity which was first described by Imerslund [L] and Gräsbeck *et al.* [8] is presented. The disease manifests itself as a megaloblastic anaemia caused by a selective malabsorption of vitamin B_{12} from the intestinal tract. The content of acid and intrinsic factor in the gastric juice is normal. The blood picture becomes normal after parenteral administration of vitamin B_{12}. Furthermore the patient has persistent proteinuria. W tried to establish the aetiology of malabsorption of vitamin B_{12} by administering intestinal juice from normal subjects, by which procedure an increased intestinal absorption

of vitamin B_{12} was demonstrated. This finding indicates the absence in these patients of a factor ("releasing factor"?) which is normally present in the intestinal juice and which is of importance for the absorption of vitamin B_{12}. Our present knowledge as to the absorption of vitamin B_{12} is reviewed briefly and a theory is advanced, partly founded on experiments, to the effect that vitamin B_{12} is normally absorbed in a peptide-bound form.

Acknowledgement

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References

1. Brøchner-Mortensen, P. and Imerslund, O.: Studies of patients with familial vitamin B_{12} malabsorption. Acta Haemat 30: 340, 1963.
2. Booth, C. C. and Mollin, D. L.: The site of absorption of vitamin B_{12} in man. Lancet I: 18, 1959.
3. Clark, A. C. L. and Booth, C. C.: Deficiency of vitamin B_{12} after extensive resection of the small intestine in an infant. Arch Dis Childh, 35: 595, 1960.
4. Collin, E., Grasbeck, R. and Kantero, W.: Studies of patient with selective deficiency in absorption of vitamin B_{12}. Blood 18: 48, 1961.
5. Cooper, B. A. and Castle, W. B.: Sequential mechanisms in the enhanced absorption of vitamin B_{12} by intrinsic factor in the rat. J Clin Invest 39: 199, 1960.
6. Dallmann, P. R. and Diamond, L. K.: Vitamin B_{12} deficiency associated with disease of the small intestine. J Pediat, 57: 689, 1960.
7. Glass, G. B. J.: Intrinsic factor: Properties and physiology. In Bjørkman, S. E.: Serum Haemat 1961. Munksgaard, Copenhagen 1963.
8. Gräsbeck, R., Gordin, R., Kantero, I. and Kuhlbäck, B.: Selective vitamin B_{12} malabsorption and proteinuria in young people. Acta Med Scand 167: 289, 1960.
9. Herbert, V.: The Megaloblastic Anemias. Grune & Stratton, New York 1959.
10. — Mechanism of absorption of vitamin B_{12}. Fed Proc 19: 884, 1960.
11. Herbert, V., Cooper, B. A. and Castle, W. B.: the site of vitamin B_{12}-IF complex releasing factor activity in the rat small intestine. Proc Soc Exp Biol 110: 315, 1962.
12. Imerslund, O.: Idiopathic chronic megaloblastic anemia in children. Acta Paediat Scand Suppl. 119, 1960.
13. Imerslund, O. and Brøchner-Mortensen, P.: Familial vitamin B_{12} malabsorption. Acta Haemat 30: 1, 1963.
14. Lamy, M., Besançon, F., Loverdo, A. and Afifi, F.: La malabsorption spécifique de la vitamine B_{12} avec protéinurie. Arch Franç Pediat 18: 1109, 1961.
15. Miller, G.: Intestinal absorption of vitamin B_{12}, and of a vitamin B_{12}-peptide complex in normal subjects and in patients with partial gastrectomy. Nature, 189: 33, 1961.
16. Mollin, F. S. and Heathcote, J. G.: Oral treatment of pernicious anaemia: Further studies. Brit Med J I: 225, 1961.
1 Mohr, E. B., Mansour, J. F., Conrad, J. L. and Porter, W. R.: Selective malabsorption of vitamin B_{12}. Amer J Med Sci 246: 689, 1963.
18. Nathan, von E. and Brauner, H.: Megaloblastische Anämie mit Relapsen ohne Achylia gastrica im Kindesalter. Ann Paediat 178: 47, 1952.
19. Reizenstein, P. G.: Vitamin B_{12} metabolism. Acta Med Scand, Suppl 347, 1959.
20. Rendick, R. H., Colman, R., London, A. and Richter, H.: Abnormal Schilling test corrected by intestinal juice. New Engl J Med, 266: 976, 1962.
21. Rovtveit, C. J.: Megaloblastisk anaemi hos et 3-års barn. Ugeskr Læg, 125: 1144, 1963.
22. Spurling, C. L., Sacks, M. S. and Jiji, R. M.: Juvenile pernicious anemia. New Engl J Med, 271: 995, 1964.

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Bacterial Metastasis Following B C G Vaccination

by ALF WALLERSTRÖM and HERBERT ENELL

When B C.G bacteria are deposited in or under the skin, they disseminate rapidly throughout the body- in animals they have been demonstrated in the internal organs within an hour of such vaccination [-1 23 27] and in human beings well defined scattered foci of proliferating epithelioid cells can be seen in parenchymatous organs within 6 weeks [8] these lesions develop rapidly within the first 2–3 months of vaccination, after which they persist un changed for 6–7 months and then slowly regress [22]. Though the foci almost regularly regress, they are doubtless the origin of the rare metastatic phenomena seen after B.C.G vaccination.

About 10 cases of fatal systemic B.C G infection are on record, including 6 from Scandinavia [3, 4, 6, 10 17 4]. Compared with the number of persons inoculated (over 100 million) this f gure is exceedingly small. The deaths could hardly be ascribed to an increase in the virulence of the B C.G.-strains used because other persons vaccinated with the same batch of vaccine and on the same occasion had reacted in a normal w y

A less widely known but possibly more common, complication is skeletal metastasis, instances of which have been reported from *inter alia* Scandinavia and Finland [1 9 11 18 26] in one case the foci were multiple; in the others solitary and located in the femur ulna, or talus. Tubercle bacilli with all the characteristics of B C.G were cultured from the lesions in each case. Fellander [5] has published a series of skeletal lesions in 1–4-year-old children vaccinated during the neonatal period. The histological diagnosis in all 10 cases was tuberculosis, and in one of the cases in which material also was cultured, tubercle bacilli of B C G -character were demonstrated. In most cases the lesions were situated in the region of the meta physis of the long bones; in the other cases, in the talus, cuboid bone or sternum.

From a bacteriological point of view the identification of B C G is not easy B C G is an originally bovine tuberculous strain that has been attenuated by culture on a special medium. When grown at 37°C on Löwen stein medium the colonies resemble those seen in cultures f the human rather than the bovine type f tubercle bacillus. When in oculated into guinea pigs B C G produces no reaction or at most slight infiltration t the sit of injection. Identification of the bacillus requires special tests: like other bovine trains, B.C G forms only an insignificant amount of nicotinic acid (niacin) in egg media (Peknice and Asmus-Garschagen

tests negative) it produces little nicotinic acid amidase and is inhibited by certain substances (thenoyl hydrazine furfuroyl hydrazine) to which the human and INH resistant bovine strains are unsusceptible like the anonymous mycobacteria. From the latter group (which produces no reaction in guinea-pigs either) B.C.G differs by its formation of pigment which is the same in the light as in the dark, by its rate of growth and temperature requirement, which are identical with those of other tubercle bacilli; certain B.C.G.-strains resemble the anonymous mycobacteria in that the oxidative metabolism is stimulated only slightly by salicylic acid derivatives (salicylate effect test 20 or less) (13). In its original state however B.C.G. is INH-sensitive and its *most specific property is, perhaps, its very* low dehydrogenase activity which is reflected in its inability to discolour methylene blue solution under anaerobic conditions within 24 hours (anonymous and saprophytic mycobacteria discolour such solutions within 1 hour while virulent tubercle bacilli require a period between these two extremes). No tests can prove that a given tubercle bacilli strain is B.C.G.; the diagnosis is made on the basis of a combination of properties, and, as a rule the bacteriological report runs: present methods cannot distinguish the strain from B.C.G.

Below 2 cases are reported in which B.C.G. vaccinated infants developed abscesses from which tubercle bacilli could be cultured with all of the typical properties of B.C.G. The diagnosis was thus *B.C.G. metastasis.*

Case Reports

Case 1. Boy, born on December 23, 1962. Mother's cousin dead of tuberculosis in 1933 and mother's uncle operated upon for tuberculosis of the lungs in 1954. Normal delivery after normal pregnancy, birth weight 2950 g. As is the rule in Sweden, he was B.C.G. vaccinated intracutaneously in the neonatal period with the generally used Swedish B.C.G. vaccine (No. 47, 0.1 ml). At the age of one month a small reaction was noticed on the vaccination spot in the left thigh, and a pea-sized adenitis in the groin. A tuberculin test at the age of three months showed a moderate to strong positive response. Other children vaccinated with the same vaccine at the same time had no complications. The patient developed normally and was quite healthy until the age of ten months. Then swelling of the left foot was observed; the skin of the dorsum pedis was red, shiny and tense, and the foot itself was warm and very sore. He was slightly febrile. Radiographic examination showed advanced changes of the 1st metatarsal bone, which was interpreted as osteomyelitis. An incision was made and a slight amount of pus was obtained. *Ordinary* culture proved negative and penicillin treatment produced no change in the patient's condition. E.S.R. (micro) was normal throughout his stay in the hospital. The Mantoux reaction 1:1000 was positive (10×10 mm), the B.C.G.-mark small and free from reaction. Radiography of the chest showed nothing remarkable.

A biopsy specimen was taken, part of which was sent for specific culture on Löwenstein's medium and the guinea-pig test. A second sample of pus for culture was taken 4 weeks later. The histological picture suggested tuberculosis. Both samples gave growth of acid-fast rods, which on further examination (see below) showed all the characteristics of the Swedish B.C.G.-strain.

Antituberculous therapy with INH and PAS was started and continued for 6 months; after an interval of 3 months, treatment was resumed for a further 3 months. The lesion healed rapidly without complications (Fig. 1–3). The boy walked without trouble at the age of 15 months and has developed normally in every respect.

On repeated sampling the serum electrophoresis was principally normal, apart from a relatively low gammaglobulin value with regard to the age (0.45–0.48 g/100 ml). Immuno-electrophoresis showed total lack of γ_{1A}-line. *He had no granulocytopenia or*



Fig. 1. B.C.G.-lesion in left metatarsale I I boy aged 10 months (Case No. 1). Intense ostitis with swelling of surrounding tissue. (Oct. 22, 1963).

lymphocytopenia and has not so far been more sensitive to infection than other children. Last control at the age of 2½ years revealed nothing of interest.

Case 2. Girl, born on November 4 1961 No known family history of tuberculosis. Normal delivery after normal pregnancy B.C.G vaccinated intracutaneously in left thigh during the first week of life at the maternity ward. The vaccination lesion healed without complications. On September 27 1963, the patient was said to ha fallen and injured her right wrist, for which medical advice was sought. Radiography showed no skeletal injury Some time later the patient complained of increased swelling of the right lower arm, the skin over the distal part was reddened and exfoliating; but the movements of the right hand appeared normal and there was not derness over the swelling On December 4 1 3 the swelling was the size of a hen's egg and fluctuant. Radiography revealed no bone lesion and no periosteal reaction. E.S R. (micro) 21 mm/1 hour Hb 11.5 g/100 ml R B C 4.6 mill. W B.C. 7 600 Diff.count N 71 E., B., M. 4 L. 23, plasma cells Mantoux reaction 1: 1000 was pos tive (10 10 mm), and the radiogram of the hest was normal. On puncture and later excision of the abscess pus and necrotic material were obtained. Pathologist report. necrotic tissue containing fragments of small essels, numerous red blood cells and leucocytes, necrotic parts surrounded by granulation tissue with epitheloid cells and giant cells. Culture on Löwenstein medium gave growth of acid



Fig. 2. Same lesion, 3 months later after curettage (Jan. 20, 1964). Strong periosteal reaction, sclerosis and enlargement of metatarsale I.



Fig. 2. Dec. 3, 1964: the lesion in metatarsale I has healed. Slight enlargement, and outline of the bone still somewhat irregular. No rarefaction.

fast rods, the guinea pig test was negative. On examination (see below) at special laboratories the strain was found to react in all respects like B.C.G.

After excision of the lesion, the lower arm was immobilised in plaster for 3 weeks, after which a very small ulcer persisted, which on examination a few months later appeared to have healed, according to the parents the small abscess had occasionally discharged pus and then closed rapidly. The mobility and the radiographic appearance of the wrist had been normal throughout. Chemotherapy was not given. When last seen in March, 1965, there was only a small contracted scar. Serum electrophoresis on that occasion showed a gammaglobulin content of 0.6 g/100 ml, total protein and other fractions within normal limits.

Bacteriological examination. Culture of the strains from these two cases on Löwenstein's medium gave growth of colonies rather like the human type of tubercle bacilli. The guinea-pig test was negative also in animals inoculated with culture subcutaneously. Both strains were sensitive to INH as well as to PAS, streptomycin and viomycin, but showed slightly decreased sensitivity to cycloserine (growth in the presence of 20 µg cycloserine per ml medium); a B.C.G.-strain which was cultivated from Swedish vaccine for comparison also showed slightly decreased sensitivity to this antibiotic. When examined at special laboratories, the patients' strains proved to be negative in niacid and nicotinic acid amidase tests and sensitive to theonyl 2-hydrazine and furfuroyl 2 hydrazine and did not discolour methylene-blue within 4 hours; at 22°C no growth was obtained within 3 weeks, and the salicylate effect test showed increased O-consumption of 4 to 28 %. The final report from both laboratories was that methods available could not distinguish the strains from B.C.G.

Discussion

In the discussion of the reasons why some individuals develop more or less serious complications after B.C.G. vaccination interest has been focused mainly on the possibility of a decreased resistance to bacterial infections. In several of the fatal cases, sampling on one or more occasions had shown a low gammaglobulin concentration in the blood, a finding which is, however, of doubtful significance in infants. In some cases B.C.G. has been given to children with hypogammaglobulinaemia without producing complications [14, 19, 20]; on the other hand, Bonton *et al.* [–] and recently Carlgren *et al.* [3] have reported each one fatal case of congenital hypogammaglobulinemia of the

lymphopenic type. Both infants were B.C.G vaccinated as newborns both died at the age of 9 months, one of them [ ] with ulceration at the site of vaccination, plasma cell pneumonia and scattered B C G nodules in various organs, the other [3], with pneumonia and generalized epithelioid cell granulomatosis with myr iads of acid fast rods, in all probability" B.C.G Of the two cases in the present series, samples from No 1 had repeatedly shown relatively low gammaglobulin values (0 45–0 48 g/100 ml) and immunoelectrophoresis had shown total lack of the γ_{1A}-line a rather common finding in children, but of unknown significance [25]. Also case No 3 had a fairly low gamma globulin value, viz. 0 6 g/100 ml. None of the children had lymphocytopenia. As lymphocytes and other mononuclear elements (immunocytes") play an im portant role in the formation of a tubercle, patients with agammaglobulinemia accompanied by profound lymphocytopenia are certainly more disposed to develop generalized B C.G.-itis than patients with a normal lymphocyte count. In the absence of lymphoid cells in sufficient number to wall off" the bacilli, the risk for an over whelming tuberculous infection is high in spite of chemotherapy [15]

Case No 3 is interesting in so far as radiography had revealed no lesion it is not known in which tissue the B C G metastases had occurred, possibly i locus minoris resistentiae following trauma The literature contains reports of o ly very few cases of metastases outsid the skeleton and internal organ [7 28] of Fellander' cases had a subcutaneal abscess interpreted as osteitis secondary to soft-tissue affection possibly in a lymph node [With its localisation close to a large joi t and its possible rela tion to trauma (a report which must, how ever be difficult to evaluate in infants) case No 2 is reminiscent f that described by Oster [28], and of a case seen previously (1957) at the Institution of Medical Microbiology in Lund a boy 3½ years old, who developed a fluctuant swelling below the left ankle possibly after a slight blow radiography showed no bone damage or destruction. Culture of material from the lesion gave growth of acid fast rods, which were nonvirulent to guinea pigs definite identification of the strain could not be carried out in this case, but p obably it was B.C.G

Disseminated B C G -complications should, of course be treated with chemotherapeutics including particularly INH a the most effective antibiotic against intra cellular tubercle bacilli and resting forms [12] All cases of skeletal metastases on record have been treated with surgical revision, immobilisation in plaster when the lesion was situated in an extremity and chemotherapy of varying duration. Oster case of extraskeletal metastasis [28] healed after surgical revision without chemotherapy as did the case described by Gendr ıer et al [7] and our case N 2 Though this serves to illustrate the benign char cter f the lesions, in our opinion B C G metastases are best treated with surgical r vision a d chemotherapy ir respectiv of the site of the lesion

From a diagnostic point of view it is im portant that samples be collected not only f r h t logical but also for bacteriological xamination Such samples are best taken befor chemotherapy is started because v n short chemotherapy (a few day) can

be enough to prevent growth. Since guinea pig test is negative in B C infections, both culture and the guinea p test should be done at the same time a positive culture combined with a neg ive guinea pig test often leads the exam r s thoughts to B C G

On the whole complications of B C vaccination, most cases of which have been reported from Scandinavia are unco m mon. Fatal dissemination is extremely rare skeletal metastases are also rare the 10 cases reported by Fellander [5] had been collected during a 10-year period at one of the largest special centres for skeletal tuberculosis in Sweden. Compared with the enormous number of vaccinations and taking into account the degree of protection offered by the vaccine—it has proved to reduce the morbidity from tuberculosis by about 80 % [16]—the phenomenon cannot be regarded as an argument against B C G vaccination as such.

Summary

An abscess in the left metatarsale I in a boy aged 10 months, and a subcutaneous abscess at the right wrist in a girl .. years old were found to contain bacilli with all the characteristics of the Swedish B C G strain. The histological picture was that of tuberculosis. Both children were B C G vaccinated in the neonatal period. They were found to have relatively low gamma globulin values but normal lymphocyte counts, the significance of these findings is discussed. The osteitis was treated with surgical revision and chemotherapy the subcutaneous abscess healed after surgical revision only followed by immobilization in plaster B C G metastases is a diagnostic alternative to solitary or multiple abscess formation of obscure origin in infants and young adults. The clinical course in cases hitherto reported has been benign.

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References

1 Bang J Eriksson, H C and Nielsen E. Osteomyelitis following B C G vaccination. Acta Tuberc Scand 33 203 1966
2 Boutou J Mackwarding, D and Smithells, R W B C G -dissemination in congenital hypogamma globul Brit Med J II 181, 1962
3 Carlgren L E, Hendricson L, Hansson G and Winkler P A case of agammaglobulinemia and fatal B C G dissemination. Acta Paediat Scand, 44 612, 1955.
4 Falkner, E., Lind A. and Ploman L. Fatal t berculosis from B.C G vaccination. Acta Paediat Scand, 44 19 1955
5. Fellander, M., Tuberculous osteitis following B.C.G. vaccination. Acta Orthop Scand, 33 116, 1962.
6. Gardborg O Iversen O. H. Tornberg, B J and Hesselberg, T Generalized B C G infection with fatal course in an infant Acta Paediat Scand, 51 293, 1962.
Gästrin, J and Mallorf A. C. quoted in reference No. 9
8. Gormsen H. On the occurrence of epithelioid cell granulomas in the organs of B.C.G vaccinated human beings. Acta Path Microbiol Scand, Suppl. 111 117 1956.
9 Haraldsson S. Osteitis tuberculosa fatalis following vaccination with B.C.G. strain. Acta Orthop Scand, 29 121 1959.
10 Hellström, V E. and Hård S. A fatality from B.C.G vaccination. Acta Dermatovener (Stockholm), 33. 130, 1953.

11. Ingemarsson O. and Jonsson T. Lupus vulgaris and multiple bone lesions caused by B.C.G. *Acta Tuberc Scand*, *30* 110, 1954.
12. Jensen, K. A.: In discussion, *Acta Path Microbiol Scand*, Suppl. *111* 119 1955.
13. Jubler, L.: Methods for the grouping and typing of mycobacteria. I. *Acta Path Microbiol Scand*, *50* 177 1960.
14. Kulbert K., Pedersen K. O and Wallenström, J. Drei Fälle von Agammaglobulinaemie. *Schweiz Med Wschr* *85*. 363, 1955.
15. Mataaxiotis, A. B.C.G.-fatalities: new aspects on the aetiology. Paper read at the meeting of Dansk Tuberkulosselskab & Dansk Paediatrisk Selskap in Copenhagen, May 21 1965.
16. Medical Research Council: 3rd report by The Tuberculosis Vaccine Clinical Trials Committee. *Brit Med J* *II* 973, 1963.
17. Meyer, J and Jensen K. A.: A fatal case of tuberculosis produced by B.C.G. *Amer Rev Tuberc*, *70* 402, 1954.
18. Mossman, A.: Osteomyelitis ulnae after B.C.G.-vaccination. *Nord Med*, *42* 1482, 1964.
19. Parker, R.: Hypogammaglobulinaemia and tuberculosis *Brit Med J* *II* 973, [illegible].
20. Porter, H. M.: Immunologic studies in congenital agammaglobulinemia with emphasis on delayed hypersensitivity *Pediatrics* *20* 958, 1957
21. Sprung, H. and [illegible]er, H.: Weitere Untersuchungen mit radioaktiv markierten Bakterien im Rahmen der Tuberkulose-Schutzimpfung. *Klin Wschr* *32* 186, 1954
22. Stojanov D. P. and Zakharov G. P. Über die mikroskopischen Veränderungen bei B.C.G.-geimpften Kindern *Zbl Allg Path*, *104* 466, 1963.
23. Ström, L. Studies of tubercle bacilli Calmette vaccine, labelled with radioactive phosphorus. *Acta Paediat Scand*, Suppl. *82*. 164, 1951
24. Thrap Meyer, H.: Generalized B.C.G. infection in man. I Clinical report. *Acta Tuberc Scand*, *29* 173, 1954
25. West C. D. Hong R. and Holland, N. H.: Immunoglobulin levels from the newborn period to adulthood and in immunoglobulin deficiency states. *J Clin Invest*, *41* 2054 1962.
26. Virtanen S. and Lindqvist I. Osteomyelitis of the femur caused by B.C.G. *Acta Tuberc Scand*, *41* 259, 1962.
27. Vogelsang, Th. M. and Wessteland P. Dispersion and viability of B.C.G. after intradermal injection in the guinea-pig. *Acta Path Microbiol Scand*, *48*. 169, 1960.
28. Ostek, R.: B.C.G.-abscess i knäregion. *Upsala Läk*, *114* 242, 1953.

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Body Length and Weight at Birth and One Year of Age in Different Communities in Israel

by RINA ZAIZOV[1] and ZVI LARON

The fact that genetic determinants affect body size has long been accepted [4] whereas it is only recently that the influence of prenatal and postnatal environmental factors on body weight and length have begun to be taken into consideration. There has been a great deal of discussion as to the ultimate effect of these factors upon final stature, but no definite conclusions have been reached as yet [5]

The population of Israel is composed of Jews and Arabs born in Israel as well as Jewish immigrants from a hundred countries from five continents. There is only scant data available comparing the growth and development of infants of the various communities which differ in their genetic socio-economic and cultural background.

Peller [3], in Israel in 1929 found that infants born to Jewish parents of Oriental origin weighed less than infants of European origin. In 1951 Halevi *et al.* [1] found no significant difference in birth weight among the various communities. These authors postulated that improvement in economic conditions among Oriental Jews had increased their birth weight. Birth length was not measured.

The present investigation was designed as a pilot study to compare length and weight at birth and at one year of age in various Jewish and in Arab communities in Israel in 1964.

Material

Five hundred and sixty two full term infants, born in the Sharon Hospital during the period between January to June, 1963, were studied. The infants were divided into groups according t their parents origin. The distribution of the infants in the various groups studied is shown in T ble 1 The comparison of mean body length at one year of age refers to healthy infants.

Methods

Body length and weight were measured at birth and t the age f one year The weight was measured on a scale with an accuracy up to 10 g. The length was measured as recumbent length. At birth, measurements were performed in the nursery by the same nurse. The measurement taken at one year of age were made in the regional w ll-baby clinics. The standing height of the parents was measured when possible The height of the f thers who did not come t the inter

[1] This study is part of thesis towards the M.D degree at the H brew Uni ersity Hadassah Medical School.

TABLE 1 *Distribution of infants according to the geographical origin of their parents*

Origin of parents Geographical area	Infants Number at: Birth	One Year
East and West Europe / Israeli born	1 6	85
Israeli born	39	13
Iraq and Iran	111	79
Yemen	162	123
North Africa	33	33
Israeli Arab	49	33

iew was taken from their army reserve books or from their identification cards. The history of the illnesses of the infants during the first year of life was documented from the registration files in the well baby clinics and from personal interviews with the mothers.

Results

Body weight

The weight in the various groups studied at birth and at one year of age is summarised in Table 2.

Comparison between females and males within the same ethnic group The Yemenite males are heavier than the females at birth ($p<0.01$) and at one year of age ($p<0.002$) whereas in the North African group the females are heavier than the males at one year of age ($p<0.002$). In the remaining groups the difference between females and males is not statistically significant.

Comparison between the various groups. At birth, the males of European and North African origin are the heaviest, followed in sequence by the Arabs, Iraqis and Yemenites. The Yemenite females and males weigh significantly less than the other groups ($p<0.05$). The Iraqi males weigh less than the European and North African males at birth ($p<0.05$). There is no marked difference between the various groups of females except for the Yemenite group.

At one year the North African males as well as the females are the heaviest and the Arabic males and females the lightest.

Body length

The mean length at birth and one year of age is given in Table 3.

Comparison between females and males within the same ethnic group There is no significant difference between females and males at birth. The males are longer than the females at one year of age in all ethnic groups. However the difference is statistically significant only in the following

TABLE 2. *Mean weight (g) at birth and one year of age. Various communities in Israel*

Origin of parent Geographical area	Birth: Males N	Mean	SD	Females N	Mean	SD	One Year of Age: Males N	Mean	SD	Females N	Mean	SD
East and West Europe	94	3443	139	81	3267	4 6	31	9021	1490	34	9900	473
Israeli Born	19	3401	120	31	3333	383	11	10313	450	4	1 117	496
Iraq and Iran	52	3397	140	9	3277	373	40	9470	1090	38	9429	385
Yemen	99	3308	348	63	3125	372	75	9410	338	47	8100	917
North Africa	27	3468	304	26	3243	339	20	10084	733	14	10032	499
Israeli Arabs	38	3320	413	31	3233	464	19	8433	910	14	7980	1320

Yemen, and Israel (including both native-born Jews and Arabs)

The analysed data lead to the following conclusions:

1 Mean birth weight in all communities ranged between 3135–3468 g (males—3138–3468 females—3135–3333 g) The Yemenite infants were lighter than the rest.

2 There is no significant difference in the mean birth length among the various groups, ranging between 49 7–50 9 cm with a tendency for the girls to be shorter (49 7 to 50.5 cm as compared to males, from 50.0 to 50 0 cm)

3 Mean weight and length at one year of age differs in the various ethnic groups. The mean weight in the various groups ranged between 8653 g to 10313 g in males, and between 7900 to 10 632 g in females. The mean body length in males ranged between 72.7 to 76.3 cm, in females between 0 0 to 75_ cm. The infants of the North African and Iraqi origin are the tallest followed, in a decreasing order by the Eastern and Western Europeans Yemenites and Arabs respectively

4 A significant correlation between parental height and the infant s length was found at one year of age in all communities besides the Yemenites. The effect of the father s height is greater than that of the mother *The effect of the parental height is* most pronounced in the North African group.

5 The study stresses the necessity of performing a cross-sectional and longitudinal study in the various communities in order to assess normal growth in this heterogeneous population.

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References

1 Halevi, H. F Brombeerg Y M. and Bakebeinsky A. Studies in anthropometry of Jewish infants in Palestine. *Am J Phys Anthropol, 9* 297 1931

2 McKeown T *Influences affecting prenatal* growth in man, in J M Tanner (ed.): *Human Growth.* Pergamon Press, Oxford 1960, p. 93.

3 Peller, S. Das pränatale Wachstum und die Neugeborenensterblichkeit bei Juden und Nichtjuden in ihrer Bedingtheit on Anlage und Umwelt. *Arch sexual Hyg ene und Demographie 46* 298, 1929.

4 Tanner, J M.: Genetics of human growth, *in* J M. Tanner (ed.): *Human Growth.* Pergamon Press, Oxford 1960, p. 43.

5 Visser, H. K. A.: Growth in early infancy in J J van der Werff ten Bosch and B. Haak (ed.) H. E. Stenfert Kroese, Leiden 1964.

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REVIEW ARTICLE

Haemodialysis in Paediatrics

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The relative simplicity and widespread availability of peritoneal dialysis have resulted in this technique supplanting haemodialysis as the method of choice in the management of many patients with renal failure. Nevertheless, haemodialysis is much more efficient, and it is generally agreed that this form of treatment is to be preferred in situations such as acute hypercatabolic renal failure and severe intoxications, where rapid correction of biochemical abnormalities or removal of toxic substances are overriding considerations. [3 11 13, 14 19]. Also in an occasional patient, peritoneal dialysis is impracticable because of intra-abdominal adhesions, recent surgery or extensive burns of the abdominal wall. Similar considerations apply in the selection of a method of dialysis for infants and children but doubt has been expressed as to the safety of haemodialysis in these small patients [5, 8, 17]. The main problem is maintenance of the constancy of the patient's circulatory volume when connected to the relatively large extracorporeal circulation. There are also the difficulties of obtaining adequate blood flow rates and the cannulation problems of repeated dialyses.

There have been a number of reports of haemodialysis in childhood and infancy including those of Carter *et al.*, [4] Walker *et al.*, [23] and Anderson *et al.*, [1] and we describe here our experience of thirty two haemodialyses in fourteen patients in this age group to emphasize the safety and practicability of this technique.

Method

The method, which has been fully described elsewhere [1] involved the use of one half of the Kolff disposable twin-coil artificial kidney and cannulation of the inferior vena cava via both saphenous veins or in the older children, the radial artery and a forearm vein. The dialyses were conducted with the patient on sensitive continuously recording weighing scales so that even minor changes (i.e. of the order of one gram) in overall fluid balance could be detected and corrected immediately. Frequent measurement of haemoglobin levels and packed cell volumes gave more specific reference to blood volume changes. When anaemia or overhydration complicated oliguria, ultra-filtration was used to remove body water and in the former case, allow large blood transfusions to be given. Patency of the cannulae between

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Patient	Age and Sex	Weight kg	Weight lbs	Initial blood urea nitrogen mg/100 ml	No. and duration of dialyses	Diagnosis	Results and comments.
M.B.	10 wks. M	3.4	7½	154	1 4 hrs.	Dysplastic kidneys. Pyelonephritis.	Survived for further 4 months
S.W.	11 wks. M	5.5	12	116	1 3 hrs.	Bladder neck obstruction.	Survived. Very well following surgery 9 month follow up.
M.H.	5½ mths. F	4.3	9½	111	3 mean 4 hrs.	Haemolytic-uraemic syndrome.	Survived. Very well at 3 yr follow up.
P.P.	10 mths. M	5.5	12	165	1 3½ hrs.	Urethral valve hydronephrosis.	Died 3 days later from primary renal disorder
B.G.	10 mths. M	7.7	17	156	1 3½ hrs.	Bronchopneumonia. Acute renal failure.	Died later from bronchopneumonia
F.D.	20 mths. F	9.3	20½	—	1 4½ hrs.	Imipramine poisoning.	In deep coma, required assisted respiration. Areflexic. Survived
W.E.	2 yrs. M	—	—	—	1 5 hrs.	Chlorpromazine poisoning.	Died from cerebral infarction as result of cardiac arrest prior to dialysis. Most of drug cleared.
B.O.	3 yrs. M	—	—	—	1 4 hrs.	Quinalbarbitone poisoning	Areflexia and coma. Survived.
K.K.	3½ yrs. M	—	—	233	1 4 hrs.	Lymphosarcoma with hyperuricaemia.	Died from his reticulosis. Serum uric acid reduced from 75 mg % to 30 mg %
K.A.	5 yrs. F	15.4	34	153*	6 mean 5.8 hrs.	60 % full thickness burns. Oliguric renal failure.	Entered diuretic phase. Died from pneumonia.
J.B.	6 yrs. F	17.8	39	103	3 mean 3 hrs.	Acute renal failure following repair of VSD under cardiopulmonary bypass.	Full recovery 6 month follow up.
P.M.	6½ yrs. F	19.7	43½	233	1 4 hrs.	Bladder neck obstruction	Survived. Alive at 9 months.
L.M.	9 yrs. F	—	—	153†	9 mean 4½ hrs.	60 % full thickness burns.	Never any return of renal function. Died after 40 days.
G.H.	14 yrs. F	29.5	65	180	2 mean 6 hrs.	D.L.E. and renal amyloidosis.	Preparation for operation and renal biopsy

* mean of 6 dialyses.
† mean of 9 dialyses.



Fig. 1. Levels of blood urea nitrogen and plasma bicarbonate in 10 patients at the start and finish of haemodialysis.



Fig. 3. Levels of haemoglobin in 8 patients and packed cell volume in 7 patients at the start and finish of haemodialysis.



Fig. 2. Levels of serum calcium and phosphate in 6 patients at the start and finish of haemodialysis.

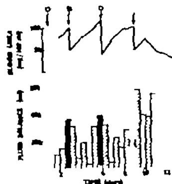


Fig. 4. J. B. aged 6, weight 17.8 kg. Acute renal failure following repair of V.S.D. under cardio-pulmonary by pass. The cross hatched columns represent fluid intake, the clear columns urine output and the blocked columns water removed by dialysis. O operation. D haemodialysis.

repeated haemodialyses was maintained by intermittent heparinisation. After the aspiration of 2 ml from the cannulae, 2 ml of a solution containing 30 mg of heparin in 100 ml of saline were injected at two hourly intervals.

Patients and Results

Table 1 summarises the clinical features and outcome of thirty two haemodialyses in fourteen children, including six infants under the age of two five of whom weighed less than 9 kg (20 lbs). Patients M.B., M.H. B.G. W.E. R.D. K.A. and L.M. have been the subject of a previous communication [1]. There were no deaths attributable to the technique and the only complication in the series was a cyanotic attack in patient B.G.

Figures 1, 2 and 3 show the changes in blood urea, plasma bicarbonate, calcium phosphate, haemoglobin and packed cell volume achieved in these patients by haemodialysis.

The results of a typical haemodialysis in a small infant are shown in Table 2.

Figure 4 summarises the illness of a child with post-operative renal failure. The volumes of water removed by ultra filtration during each dialysis are shown.

Discussion

The indications for haemodialysis in paediatric practice are similar to those for adults. However, in acute renal failure

TABLE — *Biochemical data of haemodialysis on patient S W*

Weight kg	Serum sodium mEq/l	Serum potassium mEq/l	Alkali reserve mEq/l	Blood urea nitrogen mg %	Serum calcium mg %	Serum phosphorus mg %	Serum protein g %	Hb %	P.C V
5.500	120	4.3	13	116	8.3	7.0	5.0	46	23
—	135	4.2	21	60	—	—	5.4	60	39
—	132	3.3	23	37	—	—	6.6	62	38
5.287	131	2.9	23	24	10.4	3.8	6.8	84	40

more reliance is placed on the clinical state rather than on biochemical findings, particularly when treating the very young infant The most important indication [6] is a deterioration in the patient s condition as shown by vomiting lethargy confusion, muscle twitching, convulsions and generalised oedema. A serum potassium above 7 mEq/l, an intractable metabolic acidosis and a blood urea nitrogen rising above 100 mg/100 ml are the principal biochemical indications. These values are meant only as a guide and patients should be referred to dialysis units before they are reached. Successful treatment by haemodialysis is more likely with early referral. When the cause of severe renal failure is not apparent there should be no hesitation *in using haemodialysis to gain time for* further investigation or until it has been shown that the renal lesion is irreversible Patients M.B S W and P.M. (Table 1) were tided over critical periods by haemodialysis, providing time for diagnosis, and, in the latter two cases, definitive treatment. P.M. was admitted to hospital in a critically ill condition with advanced uraemia of unknown cause He was unconscious and had a blood urea nitrogen of 233 mg/100 ml, plasma potassium of 6 6 mEq/l, plasma bicarbonate of 5 mEq /l and haemoglobin of 7 g/100 ml. Immediate haemodialysis produced a dramatic improvement and he was subsequently found to have congenital bladder neck obstruction and cutaneous ureterostomies were carried out

Repeated haemodialysis for the management of acute renal failure in the adult is well established [20 22] and has been found applicable in children as shown by patients M.H L.M. K.A. and J.B (Table 1) The difficult cannulation problems of repeated haemodialysis in the adult have been overcome by using arteriovenous shunts [16] or by repeated percutaneous catheterization of the femoral vessels [19] Here bilateral saphenous vein catheterization has proved both simple and safe and, *contrary to the view expressed* by Walker *et al.* [23] such catheters may be left in situ for up to three weeks and also be used for intravenous therapy Intermittent heparinisation of the cannulae allows mobility of the patient between dialyses and this procedure is simple enough to have been carried out on occasion by a parent of the patient. No embolic or septic complications relating to the cannulae have been encountered.

Haemodialysis was the only practicable means of treating the hypercatabolic renal

failure in patients L.M. and K.A. (Table 1) as severe burns over the abdominal walls precluded peritoneal dialysis. Repeated short haemodialyses at intervals of 36 to 48 hours maintained their blood urea nitrogen levels below 125 mg/100 ml and it is doubtful whether peritoneal dialysis, had it been possible would have been efficient enough to control their rapidly progressing uraemia An essential part of their management was the provision of a high calorie and protein intake by the intravenous route as nausea and lethargy made adequate oral feeding impossible. The frequent dialyses allowed reduction of body water by ultra filtration to accommodate the fluid volume required. Patient J.B (Table 1 Figure 4) developed acute oliguric renal failure following cardiac surgery Anorexia and vomiting precluded oral feeding and parenteral nutrition was necessary to maintain a high calorie and nitrogen intake in the immediate post-operative period. This was given as amino acid solution (Aminosol Fructose Ethanol, Vitrum) and fat emulsion (Intralipid 20 %, Vitrum). A mean volume of 820 ml of water was removed during each of the 3 dialyses to make space for this as well as the blood transfusion required.

Patients F D W E. and R O illustrate the increasing problems of acute intoxication in infants from a wide range of hypnotic and tranquillising drugs. Although imipramine [15], chlorpromazine [7] and barbiturate poisoning should be treated by forced diuresis in the first instance there should be no hesitation in resorting to haemodialysis when such an infant shows a deteriorating clinical picture and progressive deepening of coma Just as in the adult [–, 11] so in infants, if a dialysis procedure is necessary then haemodialysis is to be preferred to peritoneal dialysis as rapid removal of the offending drug is the main object of the treatment. Many of these drugs are partially protein bound but nevertheless it is still possible to dialyse out a critical amount of the drug.

When a patient has been considered too ill to survive the journey to the dialysis unit, we have been able to transport our equipment and carry out the treatment at the referring hospital Patient S.W (Table 2) underwent a successful haemodialysis at a hospital 65 miles from our base.

Our results confirm the efficiency of haemodialysis in small children. The blood flow rate is often poor at the beginning of a dialysis but improves as the treatment proceeds. Figs 1 and 3 show that the flow rates achieved have been sufficient to give adequate dialyses. Chamberlain et al [5] referring to the work of Simon & del Greco [21] state that relatively little fluid can be removed by using one coil of the Kolff kidney with blood flow rates as low as 100 ml/minute However Simon & del Greco were treating adults and in our small patients there was never any problem in removing sufficient water by ultra filtration. For example patient S W (Table) who weighed 5.5 kg (1 lbs) received a 285 ml blood transfusion as well as 120 ml of 5 dextrose to compensate for unavoidable ultra filtration, and weighed 113 g less at the end of a 3 hour dialysis. Thus if maximum dehydration had been an object of this dialysis, at least 403 ml of water could have been removed.

The main danger of haemodialysis of infants lies in the possibility of causing a drastic change in the patient's relatively

small blood volume and Chamberlain *et al* [5] have suggested that reliable control of minute to minute fluctuation in this cannot be ensured. By continuous monitoring of the patient's weight [23 1] correction of any change can be made as it occurs and in practice we have had no difficulty in maintaining the constancy of the patient's blood volume, even in infants weighing as little as 3.4 kg (7½ lbs) and 4.2 kg (9½ lbs)—patients M.B. and M.H Table 1

A second complication is the haemodialysis *disequilibrium syndrome* [9 18] due to too rapid reduction in the urea concentration in the extracellular fluid relative to that in the intracellular fluid resulting in an osmotic gradient between the two This can cause cerebral oedema which may be manifested as cyanotic attacks or generalised convulsions. The relative high efficiency of haemodialysis in these very small patients may make them especially predisposed to this complication. In a recently published series [12] three of four infants and children, dialysed for the haemolytic-uraemic syndrome, died with cerebral oedema which was attributed to too rapid lowering of the blood urea. The disequilibrium syndrome may be prevented by the use of a high bath glucose concentration—1 to 15 g/l [10] by restricting the blood flow rate to less than 100 ml/minute [1] and by undertaking dialysis whenever possible at relatively low blood urea nitrogen levels—100 to 125 mg/100 ml. In our series the complication was met with only once. Patient B G had a cyanotic attack during dialysis and here in error insufficient glucose had been added to the dialysing fluid which had a glucose concentration of only 4 g/l.

In our experience, haemodialysis of infants and small children has proved to be a simple and safe alternative to peritoneal dialysis and there need be no hesitation in using it in clinical situations where efficiency of dialysis is at a premium.

Summary

Thirty two haemodialyses were carried out in six infants and eight small children using one half of the Kolff twin-coil artificial kidney The patients weights ranged from 3 4 kg (7½ lbs) to 29.5 kg (65 lbs) The danger of producing a significant change in the patient's blood volume was avoided by continuous weight recording during dialysis. When repeated haemodialyses were required the cannulae were kept patent by intermittent heparinisation. There were no deaths or serious complications attributable to the technique and the method proved efficient in the treatment of renal failure and a variety of intoxications as well as in the correction of overhydration.

References

1 Anderson J Lee, H. A. and Stroud C. E.: Haemodialysis in Infants and Small Children. *Brit Med J* 1 1403, 1966.

2 Berman L. B. and Vogelsang, P Removal rates for barbiturates using two types of peritoneal dialysis. *New Eng J Med*, 270 77 1964.

3. Burns, R. O Henderson L. W Hager, E. B. and Merrill, J P Peritoneal Dialysis Clinical Experience. *New Eng J Med*, 267 1060 1962.

4. Carter, F H., Aoyama, S., Mercer, R. D and Kolff W J Haemodialysis in Children. *J Pediat* 51 125, 1957

5. Chamberlain, M. J., Shackman, R., Smith, E. K. M. and Wrong, O. M.: Correspondence. Brit Med J I 1610, 1963.
6. Clapp, W. M., Holmes, J. and O'Brien, D.: Extracorporeal Haemodialysis in Children. Amer J Dis Child, 104 45, 1962.
7. Dilworth, N. M., Dugdale, A. E. and Hilton, H. B.: Acute poisoning with chlorpromazine. Lancet, I 137 1962.
8. Giantonio, C. A., Vitacco, M., Mendilaharzu, J., Mendilaharzu, F. and Rutty, A.: Acute renal failure in infancy and childhood: Clinical course and treatment of 41 patients. J Pediat 61 660, 1962.
9. Kennedy, A. C., Linton, A. L. and Eaton, J. C.: Urea levels in cerebrospinal fluid after haemodialysis. Lancet I 410 1962.
10. Kennedy, A. C., Linton, A. L., Luke, R. G., Renfrew, S. and Dinwoodie, A.: The pathogenesis and prevention of cerebral dysfunction during dialysis. Lancet I 790 1964.
11. Lee, H. A. and Ames, A. C.: Haemodialysis in severe barbiturate poisoning. Brit Med J I 1217 1965.
12. Moorhead, J. F., Edwards, E. C. and Goldsmith, H. J.: Haemodialysis of three children and one infant with haemolytic-uraemic syndrome. Lancet, I 570 1965.
13. Parsons, F. M.: Indications for Haemodialysis in Acute Renal Failure, in Shaldon and Cook (ed.): Acute Renal Failure. Blackwell Sci. Publ., Oxford 1964, p. 129.
14. Prichard, A. and Smith, E. K. M.: Daily peritoneal dialysis in renal failure. Brit J Urol, 36 493, 1964.
15. Prout, B. J., Young, J. and Goddard, P.: Imipramine poisoning in childhood and suggested treatment. Brit Med J I 972, 1965.
16. Quinton, W., Dillard, D. and Scribner, B. H.: Cannulation of blood vessels for prolonged haemodialysis. Trans Amer Soc Artif Intern Organs 6 104 1960.
17. Segar, W. E., Gibson, R. K. and Rhamy, R.: Peritoneal dialysis in infants and small children. Pediatrics, 27 603, 1961.
18. Shackman, R., Chisholm, G. D., Holden, A. J. and Piggott, R. W.: Urea distribution in the body after haemodialysis. Brit Med J II 335, 1962.
19. Shaldon, S., Chiandussi, L. and Higgs, B.: Haemodialysis by percutaneous catheterisation of the femoral artery and vein with regional heparinisation. Lancet, II 857 1961.
20. Shaldon, S., Pomeroy, J., Rae, A. L., Rosen, S. M. and Shaldon, S.: Daily haemodialysis in hypercatabolic acute renal failure. Brit Med J II 407 1964.
21. Simon, N. M. and del Greco, F.: Correspondence, Lancet, I 101 1965.
22. Teschan, P. E., Baxter, C. R., O'Brien, T. F., Freyhof, J. N. and Hall, W. H.: Prophylactic haemodialysis in the treatment of acute renal failure. Ann Intern Med, 53 992, 1960.
23. Walker, J. G., Garsenstein, M., Higgs, B., Lawson, T. R. and Shaldon, S.: Extracorporeal haemodialysis in children. Arch Dis Child, 37 578, 1962.

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CASE REPORT

Foetal Exsanguination from Hemangioendothelioma of the Skin

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Blood can be lost from the foetal circulation in sufficient amount to threaten the life of the child [17]. Several causes of foetal blood loss are known. Foeto-maternal transfusion [6, 7], twin-to-twin transfusion [3, 5, 13], ruptured vasa praevia [4, 15, 22], incision of the placenta at caesarean section [16], and lesion of the placenta by amniocenthesis [23] and catheterisation [15]. In certain instances of premature separation of the placenta during labor there may be a considerable degree of bleeding from the foetal side of the placenta [10].

This paper deals with a cause of foetal exsanguination which so far as is known has not hitherto been reported.

Case Report

The child (a boy) was born at full term after a normal pregnancy. There was no Rhesus incompatibility. The birth took place in a nursing home in the country-side. It was uncomplicated and lasted for 10–12 hours. The membranes ruptured 3 minutes prior to delivery. There was a substantial amount of blood in the amniotic fluid.

The child appeared mature but was smaller than normal. Length 46 cm. Weight 2650 g. Immediately after birth, the child was shallow-breathing and flaccid. Artificial respiration and oxygen was administered. Seven minutes after birth a satisfactory cry occurred, and the child developed a more normal appearance. The placenta was normal without signs of detachment. There was neither a velamentous insertion of the cord, nor any torn vessels.

An almost circular skin tumor 4 cm in diameter was observed on the child's upper back. It was raised approximately 1 cm above the level of the skin. It was covered by skin around the edge, but for the rest it was ulcerated with hemorrhagic necroses. The tumor bled feebly approximately one hour after delivery and was later covered by thick brownish red crusts.

For the first 6 hours after birth there were no noticeable alteration in the child's condition. It then deteriorated rapidly and was transferred to Rikshospitalet, Oslo. On admission, 12 hours after birth, the child was very pale and slightly cyanotic. It appeared weak, and cried only faintly. Temperature 33.9°C. Pulse 80, regular. Small amounts of a watery, blood stained fluid were seeping out from the ulcerated surface of the skin tumor. Further general examination was normal. The hemoglobin was 9.3 g. The erythrocyte count was 2.62 million, and the colour index was 1.18. The leucocyte count was 44 000 with 37 % neutrophils, 57 % lymphocytes, 1 % monocytes, and 5 % eosinophils. The platelet count was 40 000. There were no signs of hemolysis. The Coombs' test was negative. Serum bilirubin

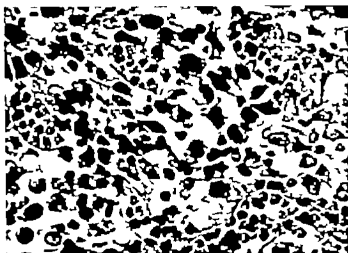


Fig. 1 H. E. stain showing capillaries and cords of tumor cells

<2 mg/100 ml. A blood transfusion (50 ml) was given with a noticeable improvement of the general condition of the child, but it suddenly expired one hour later

Necropsy findings. Apart from the skin tumor no other tumors could be demonstrated. Microscopically (Fig. 1 and 2), the tumor consisted of a sponge-like network of more or less irregular saccular sinuses enclosed by an argentaffin reticulin sheath. These lumina were lined by varying layers of large, spindle-shaped and polyhedral cells. Some of them were multinucleated. The nuclei differed in size shape and dit Many of them were monstrous with pronounced atypia and abundant mitotic figures. On the free surface of the tumor there were large hemorrhagic and necrotic areas. In some places it appeared as if the tumor had started to invade the subcutaneous fat and fascia.



Fig 2. Silver impregnation showing reticulin network and tumor cells with formation of primitive lumina.

The tumor was interpreted as a possible malignant hemangioendothelioma.

The lungs were rather airless. Abundant erythrocytes, fibrin, blood pigment and pigment macrophages were found in the alveoli and bronchioles. Moderate amounts of amniotic debris could also be seen.

Pathological changes in the brain could not be demonstrated by the neuro-pathologist.

Discussion

This child was born with a posthemorrhagic anemia due to intrauterine bleeding from an ulcerated hemangioendothelioma of the skin. It is well known that these tumors frequently ulcerate and are likely to bleed [19]. A hemoglobin of 9.5 g in a newborn indicates a serious blood loss. The aspiration of hemorrhagic amniotic fluid to the lungs may have contributed to the fatal outcome.

In a case like ours, one must take into consideration the possibility the thrombopenia-hemangioma syndrome (Kasabach-Merritt's syndrome) [10, 11]. Neither thrombopenia nor a giant tumor could, however, be demonstrated.

The appearance of the child during the first hours after birth did not raise the suspicion of a grave anemia and threatening hemorrhagic shock. Initially the condition was therefore misinterpreted as respiratory distress which rapidly subsided. This is entirely in accordance with the experience of other authors [6, 15]. Posthemorrhagic anemia which results in an exsanguinated newborn is difficult to differentiate from asphyxial shock and represents an emergency of the first order. Sometimes it is confusing that the exsanguinated baby may appear deceptively well until shortly before death [15]. Reliance on clinical features alone will result in a high mortality.

There has been considerable debate as to the definition and diagnostic criteria of the so-called hemangioendothelioma [19, 20, 21]. Stout describes two typical features of these tumors, first, the formation of atypical endothelial cells in greater numbers than required to line the vessels with a simple endothelial membrane and, second, the formation of vascular tubes with a delicate framework of reticulin fibers and a marked tendency for their lumens to anastomose [19].

Andersen [1] found that in children the hemangioendotheliomas were mainly localised to the skin. They are often present at birth [12, 14, 18, 19]. A number of infantile hemangioendotheliomas of the liver are also reported [ ]. In some cases multiple tumors in various organs may be disclosed [9, 19]. The question arises as to whether these are metastases or tumors with multicentric origin. It has been stressed by some authors that hemangioendotheliomas in children are benign even when the histological picture indicates malignancy [1, 8]. Kauffman & Stout [1] have, however, published a series of malignant hemangioendotheliomas in infants and children.

Summary

A case of intrauterine foetal exsanguination is reported. The bleeding was due to a congenital, ulcerated hemangioendothelioma of the skin.

References

1. Andersen D.: Tumors of infancy and childhood. *Cancer 4* 890, 1951
2. Arnelius, O Hemangioendotheliom i lever hos spädbarn. *Nord Med, 70* 1248, 1963.
3. Bergstedt, J Monozygotic twins, one with high erythrocyte values and jaundice the other with anaemia neonatorum and no jaundice. *Acta Paediat Scand, 46* 201 1957
4. Bergström, H.: Intrauterin fosterdöd genom Lebblodning. *Nord Med, 69* 592, 1963.
5. Betke, K.: Die Anämien des Neugeborenen. *Dtsch med Wschr 86* 64, 1961.
6. Betke, K.: Blutwerte und Blutungsanämien bei Neugeborenen. *Zbl Gynäk, 85* 113, 1963.
7. Chown B.: Anaemia from bleeding of the fetus into the mother circulation. *Lancet, I* 1213, 1954.
8. Dargeon H.: *Tumors of Childhood*, Hoeber New York 1960, pp. 263–265
9. Dorrance, J.: Multiple malignant hemangioendotheliomas in an infant. *Am J Dis Child, 55* 559 1938.
10. Good T., Carnazzo, S. and Good R.: Thrombocytopenia and giant hemangioma in infants. *Am J Dis Child, 90* 260, 1955.
11. Kasabach, H. and Merritt, K.: Capillary hemangioma with extensive purpura Report of a case. *Am J Dis Child 59* 1063, 1940.
12. Kauffman, S. and Stout, A.: Malignant hemangioendothelioma in infants and children. *Cancer 14* 1186, 1961.
13. Klingberg, W., J ... ra, B. Allen W and Dempsey E.: Placental parabiotic circulation of single ovum human twins. *Am J Dis Child, 90* 519, 1955.
14. Lieberman, E. Hypertrophic hemangioendothelioma of infancy *Am J Roentgenol, 46* 387 1941
15. Mitchell, A., A ... O and Russell, J Perinatal death from fetal exsanguination. *Brit Med J I* 611 19..
16. Nelson W E. and Gordon H H *Textbook of Pediatrics* by W E. Nelson, 6. ed., Saunders, Philadelphia 1954 p. 288.
17. Schaffer, A. J *Diseases of the Newborn.* Saunders, Philadelphia 1960, p. 12–17 1st ed.
18. Smith C.: Massive cervical hemangioendothelioma in newly born infant. *Am J Dis Child, 45* 134 1933.
19. Stout A.: Hemangio-endothelioma: A tumor of blood vessels featuring vascular endothelial cells. *Ann Surg 118* 445 1943.
20. Sutherland D Haemangio-endothelioma (Angioplastic sarcoma). *Brit J Dermatol, 70* 133, 1958
21. Sweitzer, S and Winer, L.: Hemangioendothelioma. *Arch Dermat Syphil, 31* 25 1935.
22. Vago, T and Caspi, E.: Antepartum bleeding due to injury of velamentous placental vessels. *Obst Gyn, 20* 671, 1962.
23. Zipursky A., Pollock, J Chown B. and Israels, L Transplacental foetal haemorrhage after placental injury during delivery or amniocentesis. *Lancet, II* 493, 1963.

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LETTERS TO THE EDITOR

T The Editor

Jalling et al. recently published a study of different methods for prevention of bacterial colonization of the umbilicus in newborn babies [1] One group of patients had their umbilical stump treated with Xeroform, another with Nobecutan. No difference in the efficiency of these two methods was found. A similar investigation was previously car ried out in Gothenburg [2]. Since we arrived t dissimilar result and since this dissimilar ity might be only ostensible we would like to comment upon their paper especially as our article is available only in Swedish

The important question in our study was whether or not the bacterial colonization of the umbilicus could be reduced by treatment with Nobecutan. In our group treated with Nobecutan, bacterial growth (Staphylococ-cus ureus + albus) inside the umbilicus was found in 69 of 125 infants, and in our group *treated with Xeroform 121 of 160* were colo-nized This was a statistically significant difference (χ^2 corrected for continuity [3] was 12.27 $p < 0.001$)

In contrast t this result *Jalling et al.* in a material of 116 cases concluded that "there was no significant difference between the group where Nobecutan was used and the controls

Like us, Jalling *et al* used the non para-metric χ^2 test for the statistical analysis of their material. The disadvantage of this test is that *when the material is of small size* great differences in the result of tw me-thods of treatment are mandatory A real difference may thus be concealed because the χ^2 test lacks specificity in determining significant small differences.

If one hypothetically supposes that the relationship between cases with and without growth of Staphylococcus aureus, found by Jalling *et al.*, remains unchanged when their material is increased t the same number as in our material (i.e. 285), the following ciphers will be derived.

	Xeroform	Nobecutan
Frequency of cases with growth of S. aureus	84/111	110/174

χ^2 corrected for continuity [3] will be 4.28. $p < 0.05$

Jalling et al. discuss the discrepancy be-tween their results and ours in relationship to the difference in bacteriologic methods used and in the epidemiologic situation in the two hospitals. *Is it not possible that the* difference between their results and ours might be caused by the difference in size of the two materials as indicated by the discus-sion abo e?

Hence we still find our conclusion to be valid: "It is thus possible that daily spraying with Nobecutan is a valuable *supplement to the other routines which ought to be* undertaken in the control of an epidemic caused by pathogenic staphylococci"

In discussing the risk of infection during *exchange transfusion* via the umbilical stump *Jalling et al.* (page 183) st te that w *recommended* treatment with Nobecutan as a suitable prophylactic. W quot in direct *translation from our paper* "The frequency of infections inside the umbilical stump is, even in the group, treated daily with N be-cutan, so high that exchange transfusion by means of a catheter inserted via the umbili cal *stump should not be accepted during the* third day of lif or later" Thus in this question we all seem to be in complete harmony

H -J Andersen, S E Holm, Á Nilsson
J W nberg

References

1. JALLING, B., LAGERCRANTZ, B., MYRBÄCK, K. E. and ENGSTRÖM, L.: Staphylococcus Aureus and Infections on Maternity Wards. III Bacterial Colonization of the Umbilicus and Exchange transfusions. *Acta Pædiat Scand, 43* 183, 1964.
— ANDERSEN, H. J., HOLM, S. E. and WICKBERG, J Bakteriefloran nyfödda na lar vid två olika antiseptiska behandlingsmetoder Svensk Läkartidn, *60* 99, 1963.
3. SNEDECOR, G W *Statistical Methods*. The Iowa University Press, Ames, Iowa 1956. 5th ed.

To The Editor

No statistically significant difference between treatment with Nobecutan and Xeroform was demonstrated in our series. We agree with Andersen *et al* that a bigger material might demonstrate such a difference but in such a material the relationship between cases with and without growth of S aureus *may change* The effect of Nobecutan is statistically proved in Andersen *et al.* series, but obviously of *limited practical importance*. Nobecutan did not prevent colonization of the umbilicus in 69 of 125 infants. (In our series treated with Nobecutan all but one of 71 umbilici were colonized with one or more strains of bacteria). We regard the results of treatment with Nobecutan as unsatisfactory and would prefer more effective method.

B Jalling R Lagercrantz, K E Myrbäck L. Engström

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NEW BOOKS RECEIVED

G Dhom: Die Nebennierenrinde im Kindesalter Orthologie und Pathologie. Springer Verlag, Berlin, Heidelberg, New York, 1965. 222 pages, ill. Price: DM 58.

Heiko Hertka: Das Gesicht des Säuglings. Ausdruck und Reifung. Verlag Schwabe & Co. Basel–Stuttgart, 1965. 96 pages, ill. Price: SFr 16.

B. J McKendry J D Baily: The new born, a practical guide. University of Toronto Press, Ontario 1965. 188 pages, ill. Price: US $6.50.

Herbert Bauusch, Otto R. Klimmer: Vergiftungen im Kindesalter Ferdinand Enke Verlag, Stuttgart, 1966. 418 pages, ill. Price: DM 57

W Fiebig, H. Weinland: Stoffwechsel der Galaktose und ihrer Derivate. Georg Thieme Verlag, Stuttgart, 1965 256 pages, ill. Price: DM 59.

BOOK REVIEWS

B J McKendry B A., M D and J D ailey M D., F R.C.P (C): Pediatric roblems in General Practice The Newborn Practical Guide.

University of Toronto Press 1965, 188 p 3.50.

The cooperation between obstetricians and ediatricians in maternity hospitals has sulted in considerable improvements of are of the newborns, but there still is a lot ft to be done. Now University of Toronto ress presents a book, "The Newborn" a ractical guide, written by the Canadian ediatricians McKendry and Bailey It ad resses itself to the physician who first xamines the baby" The book is well ritten, simple, interesting and handy Our nowledge about the neonatal period is increasing rapidly and it makes great demands on its performer to be the doctor responsible for a newborn baby The great experience of the authors in this field has made it possible for them to concentrate on the essential aspects of diagnosis and therapy And in spite of this concentration the book is highly readable and stimulates to further exploration of the literature Because of this one might regret the meagre number of references.

John Lind

J aku Gantrea Die richtige Ernährung der schwangeren und der stillenden Frau und ihre Bedeutung für die Gesundheit von Mutter und Kind.

Veb Gustaf Fischer Verlag, Jena 1965. 386 pp DM 81 10.

The book is a translation from the original Roumanian manuscript. It is a well written review of all nutritional factors affecting the human offspring, from the influences on the gonad cells to the passage of vitamins through the mammary glands. Special emphasis is laid on the appearance of mal formations. There are more than 1100 references to the literature from all over the world. Although these references are from

1960 or earlier this part of the book is a valuable source of information for pediatricians, obstetricians and nutritionists with interest in this field. The last chapter of the book deals with basic nutrition during pregnancy and lactation with advice for expecting mothers.

L. Soderhjelm

Rheumatic Fever Diagnosis, management and prevention. By *M Markowitz and A. G Kuttner*

Volume II in the series Major problems in clinical pediatrics. Saunders, Philadelphia and London 1965 242 p illustrated £2.12.6

New cases of rheumatic fever have become more and more rare in the Scandinavian countries during the last decade and the character of the disease has changed towards more benign forms. The same tendency seems to be present in the United States. In the beginning of the 60th, however almost one per cent of the conscripts were found incapable of military service because of rheumatic heart disease. Rheumatic fever thus still represents a very important problem and this monograph by the wellknown authors is a most valuable summary of our knowledge about this disease. Regarding the diagnosis a discussion of Wallgren's criteria is neglected. The authors are especially interested in the bacteriology and serology of the streptococci and this part is very extensive. The literature concerning prophylaxis and the more controversial hormone treatment is penetrated in an excellent and objective way In spite of the fact that the long term results hardly supports the value of hormone treatment, this is recommended in cases of a more severe character Clear and concise rules for treatment are given.

This high class monograph can be recommended for all interested representatives for adult medicine and pediatrics.

B Hellström

ANNOUNCEMENT

IVth International Symposium on Cystic Fibrosis of the Pancreas (Mucoviscidose) and second administrative session of the International Cystic Fibrosis (Mucoviscidosis) Association. Berne/Grindelwald, September 19th–22nd, 1966.

Programme 1 Chemistry of the Glucoprotein and Mucous Secretion. 2. Serous Secretion. 3. Clinical Investigations. 4. Genetics. 5 Therapy 6. Social Aspects.

Auditors limited. Admission fee sfr 50 Fee for the following arrangement sfr 180. (3 nights at a first-class hotel including all meals.)

Registration until July 1st, 1966, and informations: Dr B Fnolet Universitäts-Kinderklinik (Prof. E. Rossi), 3008 Berne/ Switzerland.

ERRATUM

The following correction should be made in the March issue (vol. 55. 2, 1966) in the paper of D Busch *et al.* Deficiency of pyruvate kinase in the erythrocytes of a child with hereditary non-spherocytic hemolytic anemia.

Page 182, column 1 first line: reduction of pyruvate kinase activity because pyruvate kinase is not a rate limiting enzyme in the glycolysis of erythrocytes [7 9].

FROM THE EDITORS

To Our Readers

Some important changes are announced on the second cover page of the present issue of *Acta Paediatrica Scandinavica*. When, in the spring of 1965 Arvid Wallgren decided to leave the post as Chief Editor his intention was also to retire from the Editorial Board. He was, however persuaded to remain as a Co-Editor for another year. That year and more has passed and it is with great reluctance that the Board of Trustees finally has to accept his definite resignation. As a sign of gratitude for his work during more than 15 years, the Board of Trustees has decided to appoint Arvid Wallgren as Honorary Editor of Acta Paediatrica Scandinavica. His successor as Co-Editor will be Bertil Lindquist who holds the chair of pediatrics at the University of Lund.

During recent years an increasing number of manuscripts dealing with problems related to applied basic sciences has been submitted for publication in Acta Paediatrica Scandinavica. The Editorial Board has become increasingly concerned with the problem of evaluating and selecting papers fairly and has come to the conclusion that a change in the character of the Advisory Board may contribute to the solution of this problem. It was suggested that members of the Advisory Board should henceforth serve a three-year term in order to make the referee system more flexible and adaptable to the rapid changes within the field of pediatrics. The members of the Advisory Board were all in agreement with these ideas. The Board has consequently been reconstituted. The names of the members for the next three years will be found on the second [illegible] page. Acta Paediatrica Scandinavica [illegible] this opportunity to thank the [illegible] members of th[illegible] old Advisory Board [illegible] their interest and coop[illegible] many years.

Th[illegible] Editorial Board has [illegible] troduce a new forum [illegible] Editor" for comments [illegible] ture short discussion [illegible] nary communications [illegible] to be important a[illegible] warrant rapid pub[illegible] has been made wit[illegible]

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Acta Pædiatrica Scandinavica 58: 546—562 Nov 1969

From the Departments of Pediatrics, Pathology I and Neurochemistry University of Gothenburg Gothenburg, Sweden

Tay-Sachs Disease

A Generalized Metabolic Disorder

by ORVAR EEG-OLOFSSON KRISTER KRISTENSSON PATRICK SOURANDER and LARS SVENNERHOLM

Tay-Sachs disease used to be regarded as a hereditary lipidosis confined to the nervous system In recent years, however increasing interest has been paid to the occurrence of foam cells and vacuolated parenchymal cells in visceral organs (9 23 ..4), but the histochemical and biochemical nature of the material assumed to be stored in these cells has never been determined

In the present clinically and pathologically typical case of Tay-Sachs disease, a biochemical examination has been performed on unfixed fresh and frozen material from brain other nervous tissue and visceral organs More than 90 % of the gangliosides from nervous tissue consisted of a ceramide monosialyl N trihexoside also termed Tay-Sach ganglioside or G_{M2} [38]. The same ganglioside occurred in spleen and liver It was isolated and its components determined

Case Report

Male infant born September 15, 1960, after an unremarkable pregnancy labor and delivery The weight at birth was 3530 g and length 50 cm. The mother aged 28 years, and the father aged 33 years, are living and well There is no known consanguinity A 5-year-old brother is alive and well. A 50-year-old cousin of the paternal grandmother and a 37 year-old cousin of the father are mentally retarded Another cousin of the father has a 7 year-old girl who is said to be mongoloid There is no other family history of convulsions, mental retardation, blindness or deaths in infancy or early childhood The parents are not of Jewish origin.

Although there was a history of drooling, excessive mucous secretions, cough, and occasional emesis with feedings, the early development of the boy was considered to be normal He was hospitalized at the ages of 3½ and 5 months for fever bronchitis and bronchopneumonia, but no abnormalities in development were noted, neurologic examination was normal and there was no hepatosplenomegaly

At the age of 7 months his parent first noticed weakness and intermittent stiffness followed shortly thereafter by loss of head control and other acquired parameters of motor development An exaggerated response to sound was noted at this time and subsequently he lost interest in grasping and playing with toys and in communicating with his environment

At the age of 12 months the weight was 9.8 kg and the head circumference 45 cm Inappropriate paroxysmal laughter and an

excess response to sound were present. He lay on his back in frog position and the feet were held in an equine position. He was unable to raise his head and could not sit. Athetoid movement were present. Neurological examination revealed generalized hypotonia and exaggerated tendon reflexes. The tonic neck reflexes and a weak Moro reflex were present as was the pharyngeal reflex. There was a normal pupillary response to light, but the child did not follow objects with his eyes and no accommodative movements were apparent. Ophthalmoscopic examination revealed macular degeneration and a cherry red spot.

At the age of 15 months convulsions of a myoclonic nature associated with unilateral or bilateral facial twitching appeared. At the age of 21 months the convulsions became generalized of a tonic-clonic type. Transitory episodes of rubor and pallor were noted at the same age.

At the age of 23 months the weight was 10.0 kg and the head circumference 51.5 cm, an increase of 6.3 cm in 12 months. The pertinent neurological findings were complete absence of environmental contact, oscillating eye movements, exaggerated responses to sound, muscular twitchings, and grand mal convulsions. The boy was now generally hypertonic with spastic reflexes, absent visual responses and absent pupillary reactions to light.

From the age of 30 months he required tube feeding and oro-pharyngeal suctioning. Frequent respiratory tract infections occurred. He assumed a permanent posture of decerebrate rigidity and responded to no stimuli. At the age of 48 months he died during a prolonged tonic seizure.

Clinical Comments

The course of the disease was typical for Tay-Sachs disease. However the boy survived longer than the usually stated age between 4 and 40 months. According to Schneck [32] one patient with Tay-Sachs disease survived to the 66th month of life.

Serial serum electrophoreses were normal but the concentration of protein bound N-acetylneuraminic acid (sialic acid) was increased. This result is in accordance with the finding by Volk *et al* [43] of a consistent elevation of globulin bound N-acetylneuraminic acid level in relation to total serum globulin. Protein electrophoresis of cerebrospinal fluid was normal at 1_ months of age but showed at the age of 23 months an elevation of α_2-globulins and a decrease of τ globulin. This latter finding suggests a decreased neuraminidase activity in CSF as τ is formed from transferrin by the enzymic release of N-acetylneuraminic acid [?7].

In the juvenile type of amaurotic idiocy lymphocytic vacuolation will appear in 20 % of the cells [29]. This phenomenon has not been described in Tay-Sachs disease. In our case we found 1 vacuolated lymphocytes, which is a normal value.

Electroencephalographic (EEG) studies in Tay-Sachs disease have been reported by several authors [5 16 28]. Repeated EEG registrations were performed by Morrell & Torres [1] and by Schneck [33]. The last author found the EEG to be within normal limits during the first 10 months of age. Between 1 and _ years of age the EEG was characterised by high voltage and slow activity with single and multiple spike potentials. After years of age both the voltage amplitude and the number of spike potentials decreased. The EEG registrations in our case were taken at the ages of 1_, 15 and 23 months, and the patterns were in accordance with the findings of Schneck.

TABLE 1 *Laboratory investigations*

Age in months	12	15	23	30	36	45
Blood						
Hemoglobin, g %	12.2	14.2	13.0	12.6	12.2	12.9
Sedimentation rate, mm/hr	16	12	19	20	22	10
Lymphocytic vacuolation, %			1			
Total serum protein, g %	6.8		6.9	7.1		
Serum electrophoresis	Normal		Normal	Normal		
N-acetylneuraminic acid, mg %				137 114	98	
Transaminases (Karmen-Ordell units) GOT/GPT	119/14		67/25			
Phosphatases (Buch Buch units) Alkalic/Acid			11/4			
Cholesterol mg %			238			
Phospholipids, mg %			260			
Glycerophospholipids, mg %			201			
Sphingomyelin, mg %			72			
Triglycerides, mg %			78			
Dye test vs. Sabin & Feldman	Neg					
Toxo-complement fixation test	Neg					
CSF						
Total protein, mg %	18.0		22.8			
Electrophoresis	Normal		$\alpha_1\uparrow$ $\downarrow$			
Urine						
Phenylpyruvic acid	Neg					
Sulfatide	Low normal value					
Electroencephalography	*A* Normal awake. Isolated suspect paroxysmal activity during sleep	*B* Marked abnormality with high voltage, slow wave activity and spike potentials	*C* As *B* but increased amount of low frequency activity with lower voltage			
Rectal biopsy				Abnormal accumulation of lipids in few ganglion cells of the myenteric plexus		

TABLE ⁹ *Tay Sachs disease Characteristics of the stored granular substances*

Method	Chemical substances	Cortical neurons	Neurons of dent. nucl.	Glial cells
PAS (McManus) frozen sections	Glycosaminoglycans and glycoproteins, glycolipids	+ + +	− +	+ + +
paraffin sections	(cerebrosides and gangliosides) and lipid pigments	−	+	+ +
Alcian blue	Acid groups (acid glycolipids and mucopolysaccharides)	+ +		+
Cresyl violet acetic acid	Metachromasia (brown-sulfatides red-gangliosides) red	+ + +	+	+
	brown	−	−	−
Scharlach R	Lipids, wide range	−	−	−
Sudan black B (frozen and paraffin sections)	Mostly unsaturated lipids	−	+	+ +
Technique of Schnabel	Cholesterol	+		( )
	Cholesterol esters	−		−
Luxol fast blue	Different lipids and (?) protein	+	+ +	+
Amidoblack	Protein	−	+ +	− +
Long Ziehl-Neelsen	Acid-fastness (lipofuscin)	−	+ −	+ +
Autofluorescence	Lipid pigments		+ +	+ +

Morphology and Histochemistry

Nervous system

The autopsy was performed 2 hours after death. The right cerebral hemisphere was taken unfixed for chemical analysis, the left one was fixed in 10 % formalin for histological examination. A large number of representative blocks were embedded in paraffin and sections of 10 μ were cut. They were examined histologically using the following staining methods: Palmgren silver technique for neurons and their axons, Luxol fast blue-cresyl violet for myelin and Nissl substance, and Holzer method for astrocytes. A number of histochemical methods (Table 2) were applied on paraffin and frozen sections. Autofluorescence of paraffin-embedded sections was studied.

Macroscopic examination. The skull was somewhat thin in the frontal region. The lepto-meninges appeared slightly thickened. The hemispheres were of equal size. Before fixation the brain weighed 1100 g. There was thrombosis of the left sinus transversus and the veins of the confluence of the left occipital lobe. The convolutions, especially of the occipital lobes, showed a moderate atrophy. There was a generalized atrophy of the cerebellar cortex. The grey matter of the hemispheres was markedly pale and reduced in thickness in the frontal and occipital regions. The border between the grey and white matter was badly defined. The lateral ventricles were slightly dilated. The white matter had a remarkably firm elastic, "rubbery" consistence and its cut surface had a watery appearance. Especially in the temporal lobes it was reduced and had a greyish colour. The appearance of the lower brain stem was slender the spinal cord and its roots showed no gross abnormality. No changes of the basal arteries were present.

Microscopic examination. Cerebral cortex. There was a considerable loss of nerve cells in all the regions of the cortex. Many of the remaining nerve cells were greatly swollen by the storage of a granular substance. In such cells the Nissl bodies had disappeared and the neurofibrils and nuclei were pushed to the periphery by this substance. In other nerve cells, no signs of storage were seen in the cell body but large amounts of granules had accumulated in balloon-shaped swellings of the axons at varying distances from the perikaryon (Fig. 1). Such swellings were



Fig. 1 Precentral gyrus. Nerve cells with granular material stored in balloon-shaped axonal swellings at varying distances from the perikaryon. Palmgren silver technique.

concentrated to the junctional area between the grey matter and the subcortical whit marrow sometimes extending for a short distance into the latter

In frozen sections the stored granules in the nerve cell bodies and the axonal swellings showed an extremely intense PAS-reaction and a weak posit ve reaction for cholesterol when treated according to Schnabel [31]. Staining with cresyl violet-acetic acid re- vealed intense red metachromasia f the granules stored in the neurons. The Alcian blue staining ga e a positive reaction, which seemed to be more intense in the axonal swellings than in the neuronal bodies. The Sudan black B reaction was negat ve or only slightly positiv

In paraffin sect ons stained with silver a delicate reticulum and light yellow granules were seen in the neuronal cytoplasm. The red metachromatic PAS- and Alcian blue-positive material seen in frozen sections was completely dissolved. Very weakly fluorescent granules staining slightly with Amido black B were seen. A moderate number of Luxol fast blue positive granules were noticed too.

Considerable marginal gliosis was seen underneath the pia. Glial cells and macrophages t nded to accumulate round the balloon-shaped neurons and the blood vessels (Fig. 2). In frozen sections, granules with staining reactions similar to those of the neuronal granules were found in these cells, but in addition, paraffin sections displayed large amounts of yellowish autofluorescent granules with staining properties consistent with lipofuscin (see Table 2 and Fig 3).

Cerebral whit matter There were severe changes f the whit matte in all regions examined The axons were less affected than the myelin sheaths, but even so showed widely spread sw lling and fragmentation. The oligodendroglial nuclei were reduced in numbe A considerable fibrous gliosis was present especially in the temporal white matter and in the internal capsule. The glial cells contained a yellow-green autofluorescent substance with the same staining reactions as those of the cort cal glial cells. Peri ascular phagocytes contained a similar substance Only a few phagocytes with su danophilic material were seen.

Basal ganglia brain st m and pinal cord



Fig. 2. Frontal cortex. Peri vascular accumulation of macrophages containing large amounts of PAS-positive material. Frozen section. PAS.

The nerve cells of the different nuclei, the spinal cord and spinal ganglia were greatly swollen by the accumulation of granules with the same proportion as those of the cortical neurons. In the pallidum, inferior olive and dentate nucleus large amounts of lipofuscin like granules were seen in addition. The brain stem and spinal cord displayed degeneration, particularly of the pyramidal tracts, lateral and anterior columns, while the posterior columns were better preserved.

Cerebellar cortex and white matter The



Fig. 3. Precentral gyrus Autofluorescent material in perivascular glial cells. The swollen neurons contain only very faint fluorescent material.



Fig. 4. Cerebellar cortex. Sharp division of the molecular layer by a horizontal band of glial fibres. Intense fibrillary gliosis in the outer lamina, which is transversed by a streak of blood vessels from the leptomeninges. Holzer

molecular layer was divided int an outer and an inner lamina of pproximately the same thickness. There was a sharp separation f these by horizontal band of glial fibres. In the outer lamina Holzer stain revealed an intense fibrillary gliosis, but only a few nuclei were detected (Fig 4) The Purkinje cells were almost completely lost and the thickness of the granular layer was extremely reduced. In frozen sections large amounts f PAS positi e material were seen in neuroglial cells and remaining nerve cells in these layers. In paraffin sections only the phagocytes took up this stain. There was an almost total destruction of the myelin sheaths and of the axons in the white matter Scarce oligodendroglial nuclei and a moderate fibrillary gliosis were present

Oculomotoric and sciatic nerves The myelin sheaths stained well with Luxol fast blue. The axons appeared to be preserved. No deposits were detected.

Visceral organs

Macroscopic findings. The heart was of normal ppearance. N theromatosis was found in the cardio-vascular system. The adipose tissue had generalized an unusual yellow colour A thrombus was found in the right pulmonary artery The lungs revealed massive bronchopneumonia. The liver and spleen weighed 650 g and 70 g respectively and were of normal appearance.

Microscopic findings Liver In paraffin embedded sections stained with routine methods no foam cells were detected A detailed re-examination of the stored unembedded liver material was performed at a time when the biochemical results were a ailable. Frozen sections f li er stored for one and a half years in 10 g formaldehyde/100 ml water and stained by the periodic acid-Schiff (PAS) technique cording to McManus showed a diffuse colour f the parenchymal cells which turned weaker after pretreatment of the sections with chloroform–methanol 2 1 v/v for two hours at +60°C This positiv staining eaction could not be attributed to preformed aldehydes since a negative result was obtained, when the periodic acid oxidation was omitted. Cryostate sect ons of unfixed li er stored for the same time as the

fixed material at −60 C and stained with the PAS technique revealed an intensely purple colour diffusely distributed in the parenchymal cells. This markedly strong reaction may partly have been due to the presence of glycogen as shown by the weaker staining obtained after pretreatment of the sections with saliva.

Since a much weaker reaction was given by the fixed material it was concluded that the glycogen of the liver cells had been dissolved during the long formalin treatment. The fading of the PAS positive staining after lipid extraction of the fixed material might indicate that in addition to glycogen diffusely distributed glycolipids were present in the parenchymal liver cells. However no storage of glycolipid in granular form as seen in the neurons was noticed in the liver cells.

There was a generalized hypertrophy of the reticuloendothelial cells. The nuclei of the Kupffer cells were large and vesicular and their cytoplasm appeared swollen. Granular material giving the yellow autofluorescence and the typical staining reactions for lipofuscin was observed in many of the reticuloendothelial cells.

Spleen, thymus, lymph nodes and bone marrow. There was considerable hypertrophy and hyperplasia of reticular cells in these organs. With the PAS method, reticular cells stained faintly positive in frozen sections. Moderate amounts of fluorescent lipofuscin-like material were seen in scattered macrophages. No foam cells were seen.

The ganglion cells of the *intestinal tract* and the *adrenal marrow* were laden with large amounts of intensely PAS positive granules in frozen sections, which were dissolved in paraffin sections. Nothing abnormal was found in the *heart, aorta, gall bladder, pancreas, kidneys, urinary bladder, testes, thyroid, skin* and *iliopsoas muscle*.

Histopathological Comments

It is well known that megalencephaly may occur in Tay-Sachs disease particularly in long-standing cases. Aronson & Volk [22] suggested that a profound astrocytosis of the white matter together with incorporated fluid causes this striking supratentorial megalencephaly. This hypothesis affords a possible explanation for the rapid increase in skull circumference observed in the present case between one and two years of age. However at the time of death the skull circumference and brain weight were within normal limits. This "normalisation" may be due to the progressing cortical destruction and severe degeneration of the white matter observed at autopsy.

The present case, like those previously published, had marked histochemical differences between cortical neurons and glial cells. In both types of cells there is a strong accumulation of PAS-positive material in frozen sections. The deposits in the nerve cells, unlike those in the glial cells, were easily dissolved by lipid extraction and revealed a red metachromasia when stained by cresyl violet-acetic acid. In Alcian blue staining the neurons reacted more strongly than the glial cells and showed a negative staining after lipid extraction. These staining results are in accordance with the findings of Diezel [8] who interpreted the PAS positive reaction of glial cells after lipid extraction as due to a firmer fixation of gangliosides to protein in these cells.

Terry & Weiss [4] have shown that the nerve cells in Tay-Sachs disease contain a characteristic electron-dense organelle called *membranous cytoplasmic body (MCB)*. According to them, the MCB are also present in the glial cells, which in addition contain numerous dense bodies made up of granules, vesicles, membranes and homogeneous material and interpreted as deri-

ved from enzymatically degraded neuronal lipids [30]. As no transitional morphologic forms between MCB and typical lysosomes were found in the neurons Wallace et al. [44] suggested that the dense bodies of the glial cells represent such transitional forms and that the final process of lipid degradation is carried out by lysosome activity of the astrocytes, macrophages and pericytes. In the present case it has been demonstrated that the glial cells and perivascular macrophages contain a substance with a primary fluorescence and histochemical properties characteristic for lipofuscin. Thus the previous observation by Diezel [8] that the glial cells contain a nonsoluble PAS positive substance is best explained by the presence of lipofuscin in these cells. It is suggested that the lipofuscin granules are located in lysosomes and contain a complex mixture of chemical substances [6] e.g. autofluorescent, polymerized, unsaturated fatty acids [36]. Provided that the accumulation of lipofuscin in glial cells and macrophages in the present case can be taken as an expression of lysosomal activity this finding may signify that the degradation of the neuronal lipids occurs in these cells, as suggested by the previous authors.

As in other published cases the cerebellum was severely affected by the neuronal storage process. In addition, the molecular layer showed a peculiar division into two laminas of equal thickness. The superficial lamina showed an intense fibrillary gliosis differing in this respect from the four cases published by Friede [10]. The occurrence of gliosis in the present case is hardly compatible with an arrested cerebellar development as suggested for the cases described by Friede

Biochemistry

Methods

Samples from the frontal and parietal lobes, cerebellum, spinal cord, spinal roots, liver and spleen were used for the quantitative lipid determinations. The brain material was separated into grey and white matter though this was greatly complicated by the cortex being diffusely demarcated and firmly attached to the white matter. The tissue was very tough and imbibed with water which poured out during the dissection. The extraction and analysis of the different lipid classes were performed with the methods recently described in detail [37]. The gangliosides were isolated from cerebral and cerebellar grey and white matter with the methods used for the isolation of infant gangliosides [36].

Lipid distribution

The results of the quantitative analyses are collected in Tables 3–4 and compared with a normal material from seven children of the same age.

Grey matter. Cerebrum. The concentration of total lipids was the same as in the normals, but the lipid pattern was changed. Total cholesterol showed normal concentration but about 8 % was in esterified form, while in normal brain only 1– of the cholesterol is esterified at this age. Total phospholipids were reduced, the decrease mainly affected kephalins and sphingomyelins, which are mainly myelin lipids [7]. There were only negligible amounts of cerebrosides and sulfatides. No reliable quantitative figures for these galactolipids were obtained, as the lipid extracts used for the hexose determination also contained ceramide oligohexosides. The concentration of lipid bound sialic acid showed a threefold and that of hexosamine a fivefold increase.

Cerebellum showed a similar pattern but its lipid content was somewhat lower.

White matter. Cerebrum. The deviations from the normal lipid pattern were much more pronounced in cerebral white matter than in the grey one. The water concentration was 90 %, which is extremely high for

TABLE 3 *Lipid composition of cerebral and cerebellar grey matter*

	Normal children (7) 3–5 yrs old Frontal lobe	Case J. A. 4 yrs Frontal lobe	Case J. A. 4 yrs Cerebellum
Water	81.1–80.4	89.3	83.8
Total lipids	28.0–3[illegible].4	8.8	2[illegible].4
Cholesterol, total	3.0– 7.0	3.9	4.4
esterified		0.41	0.12
Phospholipids	21.3–23.6	13.[illegible]	1[illegible].3
kephalins	8.5–12.2	0.7	3.8
lecithins	8.3–10	7.2	3.0
sphingomyelins	1.7– 2.8	1.2	1.6
Galactolipids	0.7– 1.6	—	—
Gangliosides	1.8– 2.0	7.3	5.
lipid NANA	0.43–0.52	1.61	1.21
lipid-hexosamine	0.171–0.200	0.92	0.[illegible]9

white matter and is of about the same magnitude as is found in foetal brain. The concentration of total lipids, calculated on a dry weight basis, was about 30%, compared to 60% for normal children of the same age. The concentration of lipids as calculated from fresh tissue weight reduces the lipid content to one-fourth of the normal value. There is thus an extreme reduction of total lipids in this case. Cholesterol constituted only 5% of dry weight matter compared to 12–15% in normals. About 8% of the cholesterol occurred in esterified form, which indicates a preceding demyelination. Total phospholipids were reduced to about 50% of the normal value for white matter; the decrease was most pronounced for kephalins and sphingomyelins. A more sensitive indicator of the loss of myelin lipids is the concentration of glycolipids other than gangliosides. In this case the concentration of non-ganglioside lipid bound hexose was about 0.4 g per 100 g dry tissue weight. Most of this hexose however was not derived from galactocerebrosides and sulfatides but from glucolipids more or less closely related to the

TABLE 4. *Lipid composition of cerebral white matter*

	Normal children (7) 3–5 yrs old Frontal lobe	Case J. A. 4 yrs Frontal lobe	Case J. A. 4 yrs Parietal lobe	Case J. A. 4 yrs Cerebellum	Case J. A. 4 yrs Spinal medulla	Case J. A. 4 yrs Spinal root
Water	0[illegible]–7[illegible] 9	90.3	89.3	86.7	77.8	81.3
Total lipids	53.6–61.9	27.3	26.7	23.9	43.7	41.0
Cholesterol, total	12.3–13.7	5.3	4.4	5.1	10.3	8.6
esterified		0.44	0.32	0.60	0.30	0.29
Phospholipids	28.0–30.8	12.8	13.6	14.3	23.3	4.3
kephalins	14.4–16.3	6.1	3.8	8.6	12.8	12.7
lecithins	8.3– 9.9	6.0	4.8	3.1	7.0	3.0
sphingomyelins	4.1– 5.2	[illegible].0	1.3	2.3	3.1	6.4
Galactolipids	12.7–13.6	0.5	[illegible].5	—	9.2	4
cerebrosides	10.3–13.8				6.8	4.6
sulfatides	1.7– 4.1				2.4	1.8
Gangliosides	0.43– 0.30	6.3	6.6	4.1	2.3	1.0
lipid NANA	0.091–0.117	1.40	1.42	0.87	0.33	0.21
lipid-hexosamine	0.041–0.033	0.[illegible]8	0.69	0.31	0.28	0.12

TABLE 5. *Brain glycolipids in normal and Tay-Sachs brain*

The figures are expressed in mg lipid per 100 g fresh tissue weight.

Compound	Normal 12 months	Normal 25 months	Tay-Sachs 48 months
Galactocerebroside	516	772	21
Galactocerebroside sulfat (sulfatide)	167	290	25
Glucocerebroside	—	—	15
Ceramide lactoside	6	7	4.8
Ceramide sialyllactoside (ganglioside G_{M3})	3	5	5.7
Ceramide N-trihexoside (asialoganglioside G_{A2})	—	—	41
Ceramide-sialyl N-trihexoside (ganglioside G_{M2})	12	10	624
Ceramide N-tetrahexoside (asialoganglioside G_{A1})	4	4	2.7
Ceramide mono-, di- and trisialyl N-tetrahexosides (gangliotetrahexosides G_1)	225	220	26

gangliosides. In a large scale isolation of glycolipids from temporal and parietal lobes, which contained both grey and white matter, only 21 mg of galactocerebrosides and 25 mg of sulfatides were found per 100 g of fresh tissue weight (Table 5). The same figures for normal brain were 500–1000 mg galactocerebrosides and 150–300 mg sulfatides.

The concentration of gangliosides in cerebral white matter, calculated on dry weight, was 12–15 times larger than in normal brains of the same age. This finding does not, however, mean that the gangliosides were mainly stored in the axons of white matter in this case. It has earlier been demonstrated [37] that the concentration of the same magnitude in the presumptive white matter as in cerebral cortex of normal infants. Because of the extreme degeneration of myelin sheaths the large increase of gangliosides in white matter is more relative than absolute. This is also evident from comparisons made on a fresh weight basis, when the increase in white matter is not more than fivefold. It is likely that the ganglioside content in the central white matter is still lower and that the gangliosides are concentrated to subcortical white matter. The microscopical examination suggested that the gangliosides were concentrated to this area, but it was not possible to separate this part satisfactorily from the rest of the white matter.

Cerebellum. Cerebellar white matter had a lipid composition, very similar to that of cerebral white matter. The accumulation of gangliosides was somewhat lower. Except for the ganglioside increase the lipid pattern of brain white matter is very similar to that seen in serious, long-standing forms of demyelinating diseases, e.g. subacute leucoencephalitis [14].

Spinal cord and roots. The biochemical signs of demyelination were also evident in spinal cord, but they were distinctly more moderate than in cerebrum and cerebellum. The ganglioside increase was also very marked in this organ. Spinal roots showed essentially the same changes as spinal cord. The concentration of gangliosides was only about 40% of that in the cord, but as spinal roots normally have a very low concentration of gangliosides, the increase in the present case was almost tenfold.

Visceral organs. Spleen and liver showed a fairly normal lipid pattern. The liver had a

slight increase of phospholipids. The concentration of gangliosides was, however increased: in the spleen it was doubled, in the liver there was a fivefold increase.

Gangliosides and related glycolipids in nervous tissue

In a previous case of Tay-Sachs disease [35] the accumulated ganglioside was found to be a ceramide monosialyl-N trihexoside (G_{M2}).

N-acetylgalactosaminoyl (1→4) galactosyl (1→4) glucosyl (1→1) N-acylsphingosine



N-acetylneuraminic acid

It comprised about 90 % of total gangliosides. In the present case, in which the disease process was still more advanced G_{M2} comprised 92 % of total brain gangliosides and the concentration of this ganglioside was about 75 times greater than in normal brains. The level of the normal major gangliotetrahexosides G_1 was considerably below normal (Table 5) Except for the large increase of ganglioside G_{M2}, the corresponding neutral aminoglycolipid, asialoganglioside G_{A2}, amounted to nearly 1/10 of the concentration of G_{M2} or about 50 mg per 100 g fresh tissue weight. Gatt & Berman [12] found that in their case of Tay-Sachs disease the ceramide-dihexosides were digalactosides, but this finding could not be confirmed here

The fatty acid compositions of G_{M2} and G_{A2} were nearly identical as were their sphingosine patterns indicating an intimate metabolic relationship. The results of the gas-liquid chromatography (GLC) analyses of fatty acids and sphingosines in the other sphingolipids are reported elsewhere [40].

The ganglioside pattern was also analysed in spinal cord and spinal roots, and in these two sources too ganglioside G_{M2} was predominant and was the only ganglioside which could be demonstrated with certainty on thin-layer chromatograms (TLC).

Gangliosides of visceral organs

The concentration of gangliosides was increased in liver and spleen. By TLC it could be shown that in addition to the normal major ganglioside, G_{M3}, of visceral organs [38] there was a second ganglioside which moved as G_{M2} from neural tissue with almost the same R_F-value in three different solvents. The two gangliosides were separated by column chromatography on silica gel columns. Ganglioside G_{M3} had the same composition as the corresponding ganglioside from spleen and liver of normal children. The new ganglioside was shown to contain glucose galactose N-acetylgalactosamine and N acetylneuraminic acid in equimolar ratios. The ganglioside was resistant to neuraminidase from *Vibro cholerae*. On acid hydrolysis and periodate oxidation it behaved as ganglioside G_{M2}, isolated from normal and Tay-Sachs brain. GLC of the liver gangliosides showed that G_{M2} contained about equal amounts of C_{18} and $C_{22}+C_{24}$ acids (Fig 5) Its fatty acid composition thus resembled a mixture of normal brain and liver gangliosides. These observations suggest that a portion of ganglioside G_{M2} in liver may have been synthesized in neural tissue and then transported to liver (C_{18} ganglioside) the other part being synthesized in liver (C_{24} ganglioside) If

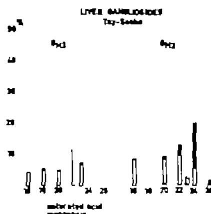


Fig. 5. The fatty acid pattern of the normal liver ganglioside G_{M2} and the Tay-Sachs ganglioside G_{M2}, isolated from the patient's liver

this interpretation of the TLC data is correct, it indicates that Tay-Sachs disease may be considered a generalized disorder of the ganglioside metabolism.

Discussion

Tay-Sachs disease is an autosomal recessive disorder appearing, as a rule among Jews. However more than 100 cases have been reported in non-Jewish children. According to Aronson [3] the incidence among Jews is 0.0002 and among non-Jews 0.000003 the carrier frequency being 0.03 and 0.003 respectively. These numbers are based on an American population.

In Sweden, only 13 cases of Tay-Sachs disease have hitherto been reported. 3 by Lindau [19] 2 by Nordlöw [22] 5 by Wallgren [45 46], 2 by Bjelkhagen [5] and 1 by Svennerholm & Zettergren [41]. The 2 cases of Nordlöw and 2 of Wallgren's cases were siblings. These 4 cases, together with a 3rd of Wallgren's and the one of Svennerholm & Zettergren, were of non-Jewish origin and derived from a rather restricted area in southwest Sweden. Our case has relatives from the same region. Of the remaining 7 cases, 4 were Jews, while the origin of the remaining 3 is unknown.

Consanguinity is common in genetic disorders such as Tay-Sachs disease. The rate of consanguinity in order of cousin marriages, is, for affected Jewish families 1–3.5% and for affected non-Jewish families 4–16% [1]. This is in accordance with the postulation of an inverse relationship between gene frequency and incidence of consanguinity. This increases the possibility that there is a relationship between the 4 families from southwest Sweden.

The most remarkable histological finding was that of axonal swellings containing a granular substance with the same staining properties as ganglioside. Such swellings have previously been seen in advanced cases of Tay-Sachs disease [ ] and in a case, clinically and histologically interpreted as Niemann-Pick disease [16]. In the present case such swellings were widely distributed and particularly frequent in the deeper layers of the cerebral cortex extending for a short distance in the subcortical white matter. Furthermore many of these swellings were located in axons of nerve cells in which no lipid deposits could be detected in the perikaryon. Considering that the chemical investigation showed a large increase in the ganglioside content of the white matter these histological findings may indicate an axonal transport of the gangliosides from the cortex to the white matter. This is in accordance with the observations of Aronson & Volk [ ] who noticed that in the later stages of the disease a decreasing concentration of the grey matter neuro

minic acid coincided with a progressive increase of this substance in the white matter

Histologically one cannot determine whether the severe affection of the white matter is due to a dysmyelinating or a myelinoclastic process. The extensive degeneration and loss of neurons, combined with the chemical finding of significant amounts of cholesterol esters in the white matter supports the opinion that the changes in the myelin sheaths are mainly secondary to axonal destruction.

The results of the chemical investigations on the spleen and liver indicate a primary disturbance in the ganglioside metabolism of these organs. Histologically there were no foam cells and histochemically no obvious signs of glycolipid storage in the lymphatic organs, bone marrow and liver The generalized hypertrophy of the reticuloendothelial cells of these organs and their content of moderate amounts of lipofuscin were striking findings. However considering the severe infection and the emaciation of the patient in the terminal stage of the disease, these changes may be unspecific and do not permit any pathogenetic conclusions.

The present case is the first for which the storage of a certain ganglioside has been demonstrated in an organ outside the nervous system. It is thus evident that from a biochemical point of view Tay-Sachs disease is a generalized metabolic disorder although the manifestations are much less pronounced generally than in the nervous system which is rather natural, since these types of gangliosides are predominantly confined to the nervous system

In another form of amaurotic idiocy described as temic late infantile lipidosis [13] familial neurovisceral lipidosis [18] or generalized gangliosidosis [25], the generalized nature of the involvement has been stressed. Nevertheless, Gonatas & Gonatas [13] have only described microscopic findings of cells with distended cytoplasm filled with a granular material. Since the swollen cells were stained with PAS in paraffin embedded tissues, it seems that the stored material is not gangliosides but lipofuscin or related material and the observed changes are probably unspecific [17].

O'Brien *et al.* [25] have reported the occurrence of ganglioside G_{M1} in spleen and liver as the single ganglioside in "generalized gangliosidosis The very vague description of this finding cannot be accepted as proof for the occurrence of ganglioside G_{M1} in these organs. Their inability to find the normal major ganglioside G_{M3} of these organs is also astonishing and in our opinion calls for a reinvestigation of the ganglioside concentration and pattern of visceral organs in generalized gangliosidosis.

The enzymic lesion in Tay-Sachs disease is still unknown. An accumulation of ganglioside G_{M2} might be caused by a disturbance of the biosynthesis of the normal monosialoganglioside G_{M1} because of a defect of a glycolipid galactosyltransferase [4] or of the catabolism of ganglioside G_{M2} because of a defect of a glycolipid β-N-acetylgalactosaminidase (Fig. 0)

Roseman and coworkers [4] have recently shown that the Tay-Sachs ganglioside (G_{M2}) is the optimal substrate for a galactosyltransferase isolated from chicken brain In this reaction a ganglioside was formed with the same chromatographic

14. Hagne, L., Sourander, P and Svennerholm L.: Unpublished observations.
15. Jorgensen L. Blackstad T W., Harkmark, W and Steen J A Niemann Pick disease. Report of a case with chemical evidence of neuronal storage of acid glycolipids. *Acta Neuropath 4* 90 1964.
16. Korey S. R. and Terry R. D Studies in T y-Sachs disease. I. B. Clinical and pathological descriptions. *J Neuropath Exp Neurol, 22.* 10 1963.
17 Kristensson K. and Sourander, P The occurrence of lipofuscin in inherited metabolic disorders affecting the nervous system. *J Neurol Neurosurg P ychiat* In press, 1966.
18. Landing B. H., Silverman F N Craig, J M., Jaco y M. D Lahey M E. and Chadwick, D L.: Familial neurovisceral lipidosis. *Amer J Dis Child, 108* 303, 1964
19. Lindau A.: Neuere Auffassungen uber die Pathogenese der familiaren amaurot schen Idiotie. *Acta Psychiat Neurol Scand 5* 174, 1930
20 Mehl, E. and Jatzkewitz, H.: Evidence for the genetic block in metachromatic leucodystrophy *Biochem Biophys Res Commun, 19* 407 1965
21 Morrell, F and Torres, F Electrophysiological analysis of a case of T y-Sachs disease. *Brain 83* 213, 1960
22. N rdlöw W A case of infantile amaurotic family idiocy with fundus changes of the ey as primary symptom. *Acta Ophthal, 24* 177 1946.
23. N rman R. M., Urich, H., Tingey A. H and Goodbo y R. A. Tay-Sachs disease with visceral involvement and its relationship to Niemann-Pick disease. *J Path Bact, 78* 409 1959
24 Norman R. M., Tingey A. H., Newman, C. G H. and Ward S. P T y-Sachs disease with visceral involvement and its relation to gargoylism. *Arch Dis Child, 39* 634 1964.
25. O'Brien, J S., Stern M. B Landin B. H., O Brien J K. and Donnell, G N Generalized gangliosidosis. *Amer J Dis Child, 109* 338, 1965.
26. Pearse, A. G. E. *Histochemistry* Churchill, London, 1960, p. 661 2nd edition.
27 Peiffer, D and Stupp L.: Die Fraktion im Liquor Cerebrospinalis. *Klin Wschr 38* 109, 1960
28. Radermecker, J Relations anatomocliniques et électroencéphalographiques dans le groupe des idioties amaurotiques. *Deutsch Z Nervenheilk 169* 238, 1952.
29. Rayner, S.: *Juvenil amaurotic idiocy in Sweden.* A publication from the Institute for Medical Genetics of the University of Uppsala, Sweden, 1962.
30. Samuels, S. Korey S. R., Gonatas, J Terry R. D and Weiss, M.: Studies in T y-Sachs disease. IV Membranous cytoplasmic bodies. *J Neuropath Exp Neurol, 22.* 81 1963
31 Schiffer, R.: Eine topochemische Methode zur Differenzierung des freien und veresterten Cholesterins. *Acta Histochem 18* 161, 1964.
32. Schneck, L.: The clinical aspects of T y Sachs disease. In *Tay-Sachs Disease* Grune & Stratton, New York, 1964 p. 21.
33. —The early electroencephalographic and seizure characteristics of Tay-Sachs disease. *Acta Neurol Scand, 41* 163, 1965.
34. Svennerholm, L.: The nature of the gangliosides in Tay-Sachs disease. In *Cerebral Lipidoses.* Black ell Publ., Oxford, 1957 p. 139
35. — The chemical structure of normal human brain and T y-Sachs gangliosides. *Biochem Biophys Res Commun, 9* 436, 1962.
36. — Chromatographic separation of human brain gangliosides. *J Neurochem, 10* 613, 1963.
37 — The distribution of lipids in the human nervous system. — I. Analytical procedure. Lipids of foetal and newborn brain. *J Neurochem, 11* 839 1964.
38. — The gangliosides. *J Lipid Res 5* 145, 1964
39 — Lipidoses. In *Metabolism nd Physiological Significance of Lipids.* John Wiley and Sons Ltd., London, 1964, p. 553.
40 — The composition of the lipophilic portion of gangliosides from normal and T y Sachs brains. In manuscript 1966.
41. Svennerholm, L. and Zetterqvist L.: Infantile amaurotic idiocy Macroscopical and chemical investigation of one case. *Acta Path Microbiol Scand, 41* 127 1957
42. Terry R D and Weiss, M.: Studies in T y-Sachs' disease. II. Ultrastructure of the cerebrum. *J Neuropath Exp Neurol, 22* 18, 1963
43 V lk, B. W Aronson S M. and Saifer, A.: The serum neuraminic acid distribution. *J Lab Clin Med, 50* 26, 1957
44. Wallace, B. J Volk, B. W and Lazarus, S S.: Fine structural localization of acid phosphatase activity in neurons of Tay Sachs disease. *J Neuropath Exp Neurol, 23* 676, 1964
45 Wallgren A.: Två fall av amaurotisk idiot. *Nord Med, 16* 3153, 1942.
46 —Fall v amaurotisk idioti. *Nord Med, 33* 45 1947

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The Thrombotest on the First Day of Life

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It is well known that the clotting power of the newborn infant s blood does not reach adult levels until several days after birth. If the infant is healthy this tem porary deficiency is not important but should it be injured during birth or be ill in any way this tendency to bleed may be dangerous. It is important, therefore to know the clotting power of a sick infant s blood so that any deficiency present can be treated. Consequently a simple reliable bedside test of blood coagulation is needed. Of all the tests available for this purpose, the thrombotest Owren [5] seems the best to use. It estimates more factors than comparable tests, also the factors which are tested are just those factors which Aballi and his co-workers [1 2, 3] showed to be reduced in the neonatal period.

Owren devised the test in order to control anticoagulant therapy by a method which is easy quick and not expensive. Owren [5] found the test to be useful also for study of liver diseases and vitamin K deficiency states. Thrombotest is designed and adjusted to be equally sensitiv t both the intrinsic and extrinsic coagulation systems The clot ting factors of the intrinsic system are present in the circulating blood. The extrinsic system is initiated by tissue fluid containing thromboplastin. The all-in-one reagent designed by Owren [5] contains cephalin from human brain or soya bean, thromboplastin from animal organs, adsorbed bovine plasma completely freed from the four factors concerned, and calcium chloride. The mixed ingredients are lyophilised and kept in vacuum sealed ampoules. The reagent is reconst tuted with distilled water for testing capillary blood.

Furthermore, the test can be performed at the cotside and involves only a small amount of blood obtained from a heel prick. It is readily available at all times and is simple, reliable and gives reprodu cible results. The reagent used is standar dised by the manufacturers. It measures factor II (prothrombin), factor VII (proconvertin) factor IX (plasma thromboplastin component—P T C) and factor X (Stuart-Prower factor) Of the alternative tests available w have found that the Quick test may be normal when the thrombotest is low The Quick test is not sensitive to factor IX which may be depressed in the newborn period. Also, it requires laboratory facilities in common with other similar tests The simple clotting time test is often normal despite considerable reduction of clotting factors It should be noted, however that the thrombotest only measures four f ctors consequently a normal result does not exclude the possibility of other factor deficiencies.

Wefring [8] used the thrombotest to assess the coagulation status of normal babies in the first six days of life. He found that the majority of the babies studied at the age of one hour had results between 20 % and 50 % and during the next few days 16 out of 28 babies had levels less than 10 %. Vitamin K treatment by intra muscular menadione and phytonadione raised the thrombotest results. Oral phytonadione had similar effects to the intramuscular preparation and so was recommended as the drug treatment of choice Wefring [8] studied normal full term infants and found no significant difference in thombotest results between the various weight groups analysed. The present study was made to determine in addition the thrombotest results in low birth weight babies and ill infants.

Method

The method as described by Owren [5] has been followed with the halved quantities of reagent later suggested by him [6].

The test is performed in the ward using 0.25 ml of the freshly dissolved reagent carefully pipetted into a small test tube which is kept in water in a vacuum flask at 37°C for a minimum of 2 and a maximum of 30 minutes. To this is added 0 05 ml of the first blood issuing from the heel prick and the time to clotting is noted. This is converted into the thrombotest percentage by reference to the graph supplied with the reagent pack. The results should be corrected for different haemoglobin values by multiplying by a correction factor The factor is 1 0 when the haemoglobin is 100 % and rises to 1.5 when 150 %.

The present study was undertaken to determine the coagulation status as measured by the thrombotest in normal and ill infants, both full term and premature The majority of these infants were consecutive admissions to the special care baby unit and for comparison 42 normal newborn infant were studied. The tests were performed as soon as convenient after birth and in every case within the first 24 hours. At the time of the test an assessment was made of the clinical state. The results are not corrected for haemoglobin levels as this involves a second heel prick and laboratory facilities, and does not significantly alter the conclusions to be drawn from the results.

Results

The distribution of the results in all the infants is shown in Fig. 1 The most striking finding is that the majority of the results were in the low range between 0 % and 40 % with the peak between 15 % and 25 %. There were only 12 babies with results over 50 %. The normal range for newborn infants is within the range where clotting is impeded. There is a sizable group of babies with levels 10 % and under Most of these were ill and will be described in more detail later

There were 43 normal full term babies, none of whom had received Vitamin K prior to the test. Only two had levels above 35 % and just over half were in the narrow range from 16 % to 25 % There were five normal babies with levels less than 10 % (Fig. 2)

Forty-one ill full term babies were studied. The range of results was wide and lacked the peak shown by the normal babies. The histogram tends to be triangular with the higher number in the lowest range of less than 5 % (Fig 3)

The results have been separated and divided into two groups, those with levels of 10 % and less and the remainder This division is made because experience with adults receiving anticoagulant therapy

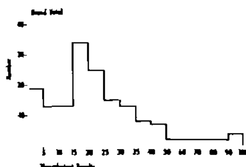


Fig. 1. The distribution of thrombotest results for all babies studied.



Fig. 2. The distribution of thrombotest results in normal and ill babies.

suggests that levels of 10% and less can be dangerous. Owren [5] in his original publication suggested 10% as the lower level of safety although Keyser [4] considered 8% to be more suitable.

There were thirteen ill full term infants out of forty-one with results of 10% and less compared with five out of the forty two normal infants with similar figures. Ten of these thirteen infants had signs of cerebral irritation. In fact of the twenty nine infants with signs referable to the nervous system nine had levels of 10% or less. Two of the four infants with proven cerebral haemorrhage had levels of 10% or less.

These infants illness may well be due to the combination of the poor clotting power and minor trauma. Many babies have minor trauma during birth and trouble develops if other factors, such as poor clotting power or anoxia are present also.

The deaths among the full term infant were confined to the group with levels of 10% and less. Four babies died—three within the first few days of birth and one eight weeks later from congenital heart disease. Two of the infants died from cerebral haemorrhage and the other infant, born by caesarean section of a diabetic mother died of the respiratory distress syndrome.

The only child who already showed signs of mental retardation had a thrombotest below 10% at birth.

The diagnoses of the ill full term infants are given in Table 1. Infants classified as having cerebral irritation are those with abnormalities of consciousness, posture tone, reactivity or movement. Infants classified as anoxic are those who had an episode of perinatal asphyxia, associated with an Apgar score of four or less and with the need for intermittent positive pressure ventilation. Those with cerebral haemorrhage had proof of the bleeding



Fig. 3. The distribution of thrombotest results in normal and ill full term babies.

TABLE 1 *Diagnoses of ill full term infants*

	Total	Thrombotest below 10%
Mild cerebral irritation	17	4
Anoxia	5	2
Cerebral haemorrhage	4	2
Haemolytic disease	4	1
Respiratory distress syndrome	4	1
Maternal diabetes	4	1
Haemorrhagic disease	1	1
Pleural effusion	1	—

TABLE 2. *Diagnoses of ill premature infants.*

	Total	Thrombotest below 10%
Cerebral irritation	17	4
Respiratory distress syndrome	15	5
Cerebral haemorrhage	3	3
Haemolytic disease	3	—
Anoxia		—
Maternal diabetes	1	—
Pneumothorax	1	—

at autopsy or lumbar puncture The first infant with haemorrhagic disease had a level less than 10% and was severely ill. She was delivered at home and within three hours of the first appearance of symptoms became exsanguinated dyspnoeic and pulseless, requiring immediate transfusion.

All the premature infants reported had received an intramuscular dose of 1 0 mg of menadione shortly after birth and always prior to the test. The normal premature infants had results less than 40%—the majority being in the 16 to 25% range (Fig 4) Only two out of the thirty two normal premature babies had levels less than 10%, compared with five out of forty two full term babies (Fig 3) The ill premature babies had a much wider range of results and whereas thirteen of the forty two were between 16% and 25% there were twelve in the range of 10% and less (Fig 4). Eight of the forty two premature infants died—seven of these were infants who had a thrombotest less than 10% on the first day The other infant who died—on the third day—with the respiratory distress syndrome did so with a level which had dropped to less than 5%. No premature baby died having a thrombotest greater than 10%. The diagnosis of the ill premature babies (Table _) shows the biggest category to be cerebral irritation. This is the group of infants who are temporarily concussed by their birth The three severely shocked babies with marked hypotonia, diminished movements and reactivity pallor and areflexia were found to have cerebral haemorrhage. In each the test was less than 5%. Two of the infants died and the third developed hydrocephalus.

A large proportion of the ill infants were babies with respiratory distress syndrome Of the fifteen babies with this illness, five died and all five had a level of 10% and less. The other baby who died weighed three pounds five ounces and was anoxic during birth, required an exchange transfusion and died with bronchopneumonia



Fig. 4. The distribution of thrombotest results in normal and ill prem ture babies.

on the third day His thrombotest of 10% on the first day had risen to 35% by the second day after the transfusion.

Discussion

The striking feature of these results is the marked contrast with adult values, confirming the results of Wefring [8] on normal newborns. The majority of the infants had results which are in the range suggested by Owren [5] as ideal for anti coagulation 10% to 30%. If correction had been made for the normal haemoglobin increase of the neonate and the maximum factor of 1.5 used for a haemoglobin value of 150 then the results in the range 10% to 25% would be from 15% to 37.5 —still low levels.

A simple explanation of the low concentration of clotting factors is that of liver immaturity The factors tested are just those manufactured by the liver and liver function is known to be depressed in the immediate neonatal period. As vitamin K often raises th thrombotest result within a day or two of birth [8] liver immaturity cannot be the sole factor Diminished liver blood flow in utero may be an aetiological factor It is possible that a low level of clotting factors serves a useful purpose in utero Placental infarction occurring primarily on the maternal side also involves the foetal side and part of the foetal placental circulation is cut off The low level of clotting factors may limit the spread of placental infarction as suggested to us by Brynmor Thomas [7]

These results suggest that many babies are at risk of prolonged bleeding In fact few babies do bleed to a degree sufficient to threaten the child life The main areas of bleeding are cranial and pulmonary It is the infant s head which takes the brunt of the force during the birth process and occasional cerebral trauma is to be expected. Minor cerebral trauma will heal spontaneously when the clotting mechanism is normal The danger arises when in addition to minor cerebral damage there is also a marked clotting defect. The main value of the test is to determine which babies are at risk. It is our routine to do a thrombotest on every infant at admission to the Special Care Unit with tests for blood glucose and pH pCO_2 etc. Those with a result less than 10% are carefully watched. If the infant has signs of cerebral irritation and a low thrombotest, he is given treatment to raise the thrombotest The initial treatment now used is phytomenadione 1 0 mg intramuscularly over and above the initial routine dose of 1 0 mg If there is no improvement in the test after four hours or the infant is seriously ill, he is given fresh compatible blood—or fresh frozen plasma 10 ml per kg

One surprising feature is the similarity of the distribution of the results of the normal full term and premature infants. As liver function is poorer in the immature child it is to be expected that the concentration of liver factors would be lower The fact that there seems to be little difference between the full term and premature infants may be due to the 1.0 mg of menadione given to the premature infants.

Summary

The thrombotest Owren has been used to assess the coagulation status of 158 premature and full term babies on the

first day of life The results were found to be low and levels of 10 % and less were found mainly in ill infants, and the majority of infants who died were in this group

The thrombotest reveals infants who are at risk of haemorrhage and also provides an easy method of monitoring treatment of coagulation deficiencies. It deserves to be used with other conventional and biochemical tests in the assessment and treatment of ill newborn infants.

References

1 Aballi, A. J., Banus, V L., de Lamerens, S., and Rozengvaig S.: Coagulation studies in the newborn period. II. *Amer J Dis Child*, *94* 589 1957
2. — Coagulation studies in the newborn period. III. *Amer J Dis Child 97* 525, 1959.
3. — Coagulation studies in the newborn period. IV *Amer J Dis Child, 97* 549 1959.
4. Keyaert, J W Control of anticoagulant therapy by thrombotest method. *Brit Med J II* 1514, 1963.
5 Owren P A.: Thrombotest, new method for controlling anticoagulant therapy *Lancet*, *II* 754, 1959
6. — Control of anticoagulant therapy *Lancet*, *I* 600, 1960
7 Thomas B. Personal communications, 1963.
8. Wefring Karl W Haemorrhage in the newborn and vitamin K prophylaxis. *J Pediat 61* 686 1962.

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Salicylate Induced Fetal Death and Malformations in Two Mouse Strains

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In studies of the damaging effect of drugs on the fetus, special interest has been focused on the actual malformations [11 28]. It nevertheless seems justified for the proposed extended drug tests, to collect more basic information about fetal death and resorption [4 6 7 20 29]

In teratological studies, drugs have been administered at an early stage of pregnancy to produce disturbances in organogenesis. The doses have been chosen empirically to induce a high incidence of malformations, but a low incidence of fetal death and resorption [27 29] The pharmacological and physiological action—including transport and distribution of th drug in the fetus—has, how ver been investigated in the late stages of pregnancy [1 23]

Salicylate derivatives seem to be appropriate for studies of fetal death t various stages of pregnancy since a considerable amount of data on these substances has accumulated from pharmacological as well as biochemical and teratological experiments [15 16 21 22, 24] The teratogenic effect of salicylates in mice has been suggested to be related to the depression of acid mucopolysaccharide synthesis [10 15 16 17].

The main aim of the present study was to investigate the influence of the time of administration on the incidence of fetal death and resorption. Moreover it was of special interest in this respect, to compare two mouse strains with different teratogenic susceptibility The present study might also be a morphological starting point for correlated pharmacological and biochemical studies.

Material and Methods

Pregnant primiparous mice were used; they were mated overnight in the following four w ys: A/Jax♂ A/Jax♂ A/Jax♀ CBA♂ CBA♀ CBA♂ and CBA♀ A/Jax♂ (T bles 1 2 and 3). In most cases, vaginal plug was easily observable on the following morning, and this day was denoted as the zero day of pregnancy [13]

Sodium salicylate 10 mg/20 g body weight in 0 2 ml of distilled w ter was gi en i.m. in single dose on one gestation day (9th 11th 13th, 15th or 17th). The fetuses from totally 120 litters were removed on the 18th gestation day and inspected for death and

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TABLE 1. *Incidence of resorbed embryos of implanted embryos from mothers given 10 mg/20 g bodyweight sodium salicylate i.m. on different gestation days*

Gestation day injected	Mating ♀ ♂	No. of litters examined	No. of implanted embryos	Resorption No.	Resorption %
9	A/Jax A/Jax	8	62	12	19
	CBA CBA	6	39	3	8
	A/Jax CBA	6	50	0	0
	CBA A/Jax	6	48	0	0
11	A/Jax A/Jax	8	60	24	40
	CBA CBA	6	33	1	3
	A/Jax CBA	6	50	7	14
	CBA + A/Jax	5	33	3	9
13	A/Jax A/Jax	6	49	33	67
	CBA CBA	5	43	3	7
	A/Jax CBA	6	41	6	15
	CBA A/Jax	6	45	3	7
15	A/Jax A/Jax	6	46	34	74
	CBA CBA	5	33	3	9
	A/Jax CBA	7	58	24	41
	CBA A/Jax	6	42	2	5
17	A/Jax A/Jax	5	33	24	73
	CBA CBA	5	38	5	13
	A/Jax CBA	6	53	13	25
	CBA A/Jax	6	40	3	8

TABLE 2. *Incidence of vessel anomalies in living embryos from mothers given 10 mg/20 g bodyweight sodium salicylate i.m. on different gestation days*

Gestation day injected	Mating ♀ ♂	No. of litters examined	No. of living embryos	Resorption No.	Resorption %
9	A/Jax A/Jax	8	50	0	0
	CBA CBA	6	38	0	0
	A/Jax CBA	6	50	0	0
	CBA A/Jax	6	48	1	2
11	A/Jax A/Jax	8	36	0	0
	CBA CBA	6	32	0	0
	A/Jax CBA	6	43	0	0
	CBA A/Jax	5	30	0	0
13	A/Jax A/Jax	6	16	3	19
	CBA CBA	5	40	0	0
	A/Jax CBA	6	35		6
	CBA A/Jax	6	42	0	0
15	A/Jax A/Jax	6	1	7	88
	CBA CBA	5	30	0	0
	A/Jax CBA	7	34	14	41
	CBA A/Jax	6	40	0	0
17	A/Jax A/Jax	5	9	0	0
	CBA CBA	5	33	1	3
	A/Jax CBA	6	39	1	3
	CBA A/Jax	8	37	3	8

TABLE 3. *Incidence of rib anomalies and vertebral anomalies in alizarin stained embryos from mothers given 10 mg/20 g bodyweight sodi m salicylate i m on different gestation days*

Gestation day injected	Mating ♀	♂	No. of litters examined	No. of living embryos examined	Rib anomalies No.	%	Vertebral anomalies No.	%
9	A/Jax	A/Jax	8	49	24	49	17	35
	CBA	CBA	6	34	18	53	4	12
	A/Jax	CBA	5	43	20	4	10	23
	CBA	A/Jax	6	46	11	24	1	2
11	A/Jax	A/Jax	7	33	1	3	0	0
	CBA	CBA	6	31	0	0	0	0
	A/Jax	CBA	6	43	0	0	0	0
	CBA	A/Jax	5	-8	0	0	0	0
13	A/Jax	A/Jax	3	13	0	0	0	0
	CBA	CBA	8	40	1	3	1	3
	A/Jax	CBA	7	43	0	0	0	0
	CBA	A/Jax	6	34	0	0	0	0
15	A/Jax	A/Jax	1	6	0	0	0	0
	CBA	CBA	5	30	0	0	0	0
	A/Jax	CBA	6	26	0	0	0	0
	CBA	A/Jax	6	39	0	0	0	0
17	A/Jax	A/Jax	3	7	0	0	0	0
	CBA	CBA	5	32	0	0	0	0
	A/Jax	CBA	6	30	0	0	0	0
	CBA	A/Jax	6	36	0	0	0	0

resorption, as well as for gross malformations [16] (Tables 1 and 2). Seven A/Jax mothers, 5 of which were mated with CBA males, gave birth before dissection on the 18th day and the fetuses could not be counted or examined. All 7 mothers had been given sodium salicylat on the 17th gestation day.

Examination for skeletal malformat ons in living embryos was performed after Alizarin red S staining [8] (T ble 3). Living embryos with exencephaly and/or gastroschisis, as well as some with ibs anomalies and some normal ones, were taken for other purposes.

Results

Resorption

The incidence of resorbed embryos is expressed as the ratio of embryos, including all degrees of resorption to the number of implanted embryos (T ble 1).

In the A/Jax strain, the incidence of resorption increased steadily from 10 % in th litters whose mothers were injected on the 9th gestation day to 4 % and 73 when the injection was made on the 15th and 17th days, respectively. Moreover two of seven mothers injected on the 17th day gave birth before dissection on the 18th day.

In the litters of A/Jax f males mated with CBA males, the incidence of resorption also increased the later in pregnancy that sodium salicylate was given. When the injection was made on th 9th gestation day no fetuses were resorbed when it was made on the 11th day the incidence was 14 %, on the 13th day 16 %, on the 15th day 41 %, and on the 1 th day -3 %. Furthermore 5 of 11 mothers injected on

the 17th day gave birth before dissection on the 18th day.

In the CBA strain, as well as in the litters of CBA females mated with A/Jax males, the resorption rate was on the contrary low even after administration in late pregnancy. Thus, the rate ranged from 0 to 13 %.

Gross malformations

A particular type of gross malformation observed was vessel anomalies [17]. They were located on the paws and in one case between the eyes. The incidence is expressed as the ratio of fetuses with this anomaly to the number of living fetuses (Table 2).

In the A/Jax strain, the vessel anomalies were found only in the fetuses of mothers injected on the 13th and 15th days, the incidence being 19 % and 58 %, respectively. In the A/Jax × CBA crossing, the vessel anomalies were observed after injection on the 13th, 15th and 17th days (incidence 6 %, 41 % and 3 %, respectively). In the CBA strain and the CBA × A/Jax crossing, the vessel anomalies were only occasionally found.

Exencephaly and/or gastroschisis were present in 6 fetuses of 6 mothers in the CBA × A/Jax crossing, after injection on the 9th–15th gestation days. One fetus of a CBA mother mated with a CBA male (injected on the 9th day) showed exencephaly. Cleft lip was occasionally observed.

Skeletal malformations

Skeletal malformations of ribs and vertebrae consisted of changes in number and of pathological fusion of these bone anlagen. The incidence is expressed as the ratio of fetuses with these anomalies to the number of living fetuses examined (Table 3).

A high incidence of skeletal malformations was found only after injection on the 9th gestation day. Rib anomalies were present in fetuses of A/Jax females mated with A/Jax males and with CBA males in about the same incidence, i.e. 49 % and 47 %, respectively. In the CBA strain the incidence was as high as 56 %, but when the CBA female was mated with an A/Jax male the incidence was only 24 %. Vertebral malformations occurred at a lower rate but in agreement with that of the rib anomalies, except in the CBA strain.

Discussion

The importance of testing teratogenic drugs for their damaging effect on the fetus in the late stages of pregnancy is clearly demonstrated in the present study. This statement is based mainly on the fact that the incidence of fetal death and resorption increased steadily the later in pregnancy that sodium salicylate was given to A/Jax mice. The difference between the strains as regards the tendency to salicylate-induced fetal death, as well as to malformation, is in agreement with earlier demonstrated strain differences concerning teratogenic susceptibility [14, 15]. Moreover, the results demonstrate the difficulty of distinguishing between the teratogenic and embryotoxic action of a drug.

Of the various drugs and substances used in experimental teratology, salicylate derivatives have been of great interest in recent years [7]. Malformations of the skeleton, blood vessels and central nervous system have been reported [15, 16, 4]. These different types of malformation oc-

respond well to the different stages of embryonic development when the drug is given, i.e., on the th up to the 13th gestation day in the mouse and rat In the present investigation the vessel anomalies of a type described earlier [17] were observed to occur in the highest incidence after injection as late as the 15th day of gestation. In addition, the strain difference between the A/Jax and the CBA strain as regards susceptibility was confirmed [15]. In the reciprocal crossings, the results demonstrate the greater risk for the embryos growing in the A/Jax mother The high incidence of skeletal anomalies after injection on the 9th day was in agreement with earlier results [15], part from the exceptionally high incidence of rib anomalies in the CBA strain. The aforementioned strain and crossing differences seem however to be supported by the other data on skeletal malformations.

It does not seem possible to ascribe the cases of exencephaly and gastroschisis to administration of salicylate.

The most interesting finding in this investigation is, however the gradual increase in incidence of fetal death and resorption after treatment from the 9th to the 17th gestation day in the A/Jax strain and in litters of A/Jax females mated with CBA males. As far as we are aware no comparable data are available, since most experiments have been made t a relatively early stage of pregnancy during which many teratogenic agents produce the highest incidence of malformations [?7] In some experiments, the rat of malformations and of prenatal mortalit during this period has been reported to vary independently whereas in others they have been closely related [*]

Lethal effects have been stated to be most pronounced at the time of implantation [10] It also seems to be the general view that when the embryo has entered the fetal period teratogenesis in the conventional sense does not occur and that any agent sufficiently potent to affect the fetus either retards growth or causes pathological changes of the types occurring postnatally [*7]

The mechanism underlying this prenatal mortality is not clearly understood. As possible sites of primary action of genetic and environmental factors which can damage the embryo Woollam & Millen [?0] have listed (a) maternal tissues, (b) placenta and (c) embryo Of these the maternal tissues and the embryo—including the fetus in the true sense—will be briefly discussed.

As far as the maternal tissues are concerned, salicylates are known to affect the organism in w ys which might secondarily affect the embryo [.1 22]. Moreover it has been shown in studies on cortisone-induced cleft palate in mice that not only the mother acting as an environmental system, but also the mother–embryo interacting system determine the final response to a drug [8] Th present results indicate that both the genetic factor and the mother as an environmental factor influence the rate of malformations and of prenatal mortality

In their list of modes of direct action on the embryo, Woollam & Millen [?0] gave the following four of which the first two seem to be of most interest.

1 Direct effect on the cell in the tructure which is to be deformed.
 Action on embryonic liver
3 Action on fetal heart and circulation.
4 A tion on embryonic endocrine system

A direct effect on the cell could produce chromosomal anomalies responsible for a deformed structure, as demonstrated with 6-amino nicotinamide in cleft palate [9]. More facts have however been assembled concerning interference with the metabolism in the tissue of maldeveloping structures [12, 14]. The decreased sulphomucopolysaccharide synthesis caused by salicylates [3, 15] is also of great interest, since mucopolysaccharides are known to assemble prior to formation of many major structures [14]. Moreover salicylates produce a decrease in energy compounds, through a depression of oxidative phosphorylation, favour protein catabolism and deplete liver glycogen stores [21]. These results have been obtained in adult systems and are not directly applicable to fetal tissues. It nevertheless seems plausible to presume that the rapidly developing embryonic tissue and its enzyme systems is more sensitive than that of the mother.

The liver is of particular interest, in view of its known role in detoxication in adults. It is not known in detail when this ability appears in the embryonic liver. However in late pregnancy this organ possesses enzyme systems capable of *in vitro* detoxication, e.g. sulphate conjugation of phenols and of steroids of phenolic or alcoholic structure [25].

Salicylates are known to elevate the plasma concentration of certain liver enzymes [18]. Although this has been interpreted as an indication of liver damage it is not known whether this applies to the embryonic liver. Salicylates are potent inhibitors of sulphate conjugations of phenols, probably in a competitive way [26].

It has been suggested that teratogenicity and lethality are only different degrees of the same reaction of the embryo to injury [27]. With respect to the fetus-damaging effect of salicylates, it is at present impossible to suggest a common mechanism for the teratogenicity and for the described variation in prenatal lethality. Moreover it is obvious that the latter effect of a tested drug is not fully taken into account in the recommended "tests for teratogenic action" [4]. It seems urgent to carry out extended investigations in different species and strains, to warrant corrections of recommendations of such drug tests.

Summary

Sodium salicylate was given i.m. in a single dose on one gestation day (9th, 11th, 13th, 15th or 17th) to pregnant primiparous mice of A/Jax and CBA strains and of their reciprocal crossings. The incidence of fetal death and resorption and of vessel and skeletal anomalies induced in the fetuses was estimated on the 18th day of pregnancy.

Fetal resorption rate increased steadily the later sodium salicylate was given in pregnancy in the A/Jax strain, as well as in the litters of A/Jax females mated with CBA males. In the CBA strain and in the litters of CBA females mated with A/Jax males, on the contrary, the resorption rate was low even after injection in late pregnancy.

Vessel anomalies were observed in the highest incidence after injection on the 15th gestation day, whereas anomalies of ribs and vertebrae showed the highest incidence after injection on the 9th day. In these respects as well the A/Jax strain as A/Jax females mated with CBA males were observed to be the most susceptible.

The present results indicate that in the suggested drug tests for teratogenic action, the drug should also be given after the organogenetic period, and that special attention should be focused on fetal lethality. The mechanisms underlying the salicylate-induced fetal damage are discussed.

References

1. Baker, J. B. E.: The effects of drugs on the fetus. *Pharmacol Rev, 12* 37 1960.
2. Blattner, R. J.: Comments on current literature. Teratogenic action of salicylates in mice. Effect of mucopolysaccharide synthesis. *J Pediatrics, 60* 110.., 1963.
3. Bostrom, H. and Mansson B.: The action of salicylates and related compounds on the sulphate exchange of chondroitin sulphuric acid. *J Pharm* (Lond), *7* 185, 1955.
4. Davey D G.: The study of the toxicity of potential drugs—base principles. A supplement to Proceedings of the European Society for the Study of Drug T xicity *International congress series* N 97 Vol VI 1965. *Excerpta Medica Foundation.*
5. D wson, A. B.: A note on the staining of the skeleton of cleared specimens with Alizarin red S. *Stain Technol, 1* 123, 19.6–28.
6. Editorial: Safety of drugs. *Brit med J 11* 48, 1962.
7. Editorial: Salicylates and congenital malformations. *Brit med J I* 332, 1963.
8. Fraser, F C., Walker, B. E. and Trasler, D. G.: Experimental production of congenital cleft palate; genetic and environmental factors. *Pediatrics, 19* 782, 1957
9. Ingalls, T H., Ingenito, E. F and Curley F J Acquired chromosomal anomalies induced in mice by injection of ter atogen in pregnancy *Science, 141* 810, 1963.
10. Jacobson B., Boström, H. and Larsson K. S.: The effect of sodium salicylate on hexosamine synthesis in eviscerated mouse fetuses. *Acta Chem Scand 18* 818, 1964.
11. Kalter, H. and Warkany J Experimental production of congenital malformations in mammals by metabolic procedure *Physiol Rev 39* 69, 1959
12. Kochhar, D M. and J mason E. M Morphological and autoradiographic studies of cleft palate induced in rat embryos by maternal hypervitaminosis A *J Embryol exp Morph, 14* ..23, 1965
13. Larsson K. S.: Studies on the closure of the secondary palate III Autoradiographic and histochemical studies in the normal mouse embry *Acta Morphol Neerl Scand, 4* 349 1962
14. — Closure of the secondary palate and its relation to sulpho-mucopolysaccharides. *Acta Odont Scand,* Suppl 31 196..
15. Larsson K. S. and Boström, H.: Teratogenic action of salicylates related to the inhibition of mucopolysaccharide yn thesis. *Acta Paediat Scand 54* 43, 1965.
16. Larsson, K. S Boström, H. and Ericson B.: Salicylate-induced malformations in mouse embryos. *Acta Paediat Scand 5* 36, 1963.
17. Larsson K. S., Ericson B. and Boström, H.: Salicylate-induced skeletal and eel malformations in mouse embryos. *Acta Morphol Neerl Scand, 6* 35, 1964
18. Manso, C., Taranta, A. and Nydick, I Effect of aspirin administration on serum glutamic oxaloacetic and glutamic pyruvic transaminases in children. *Proc Soc exp Biol* N Y *93* 84, 1956.
19. Murphy M. L.: Factors influencing teratogenic response to drugs. Published in *Teratology Principles and Techniques.* The Uni ersity of Chicago Press, Chicago and London, 1965.
20. Robson J M. The action of drugs on the mbryo Pharmacological considerations. *Proc Roy Soc Med 46* 900, 1963.
21. Smith, M. J H Salicylates and metabolism. *J Pharm Pharmacol, 11* 05, 1959.
22. Smith, P K. The pharmacology of salicylates and related compounds. *A NY Acad Sci, 86* 38, 1960.
23. Ullberg, S. Specific localization of labelled hormones and vitamins whole-body autoradiographic observations In *Isotopes in Experimental Pharmacology* (L. J Roth, ed). University of Chicago Press, Chicago, 1965, pp. 63–74.
24. Warkany J and Takacs, E Experimental production of congenital malformations in rats by salicylate poisoning. *Amer J Path 35* 315, 1959
25. Wengle, B Studies on ester sulphates. 21 On sulphate conjugation in foetal human tissue extracts. *Acta Soc Med Upsalien, 69* 105, 1964.
26. — Personal communication 1966.
27. Wilson J G Embryological considerations in teratology Published in *Teratology Principles and Techniques* The Uni ersity of Chicago Press, Chicago and London, 1965.

28. WILSON J G and WARKANY J *Teratology Principles and Techniques*. The University of Chicago Press, Chicago and London, 1965.

29. WOOLLAM, D H. M. and MILLEN J W The action of drugs on the embryo Experimental mammalian teratology and the effect on the embryo. *Proc Roy Soc Med*, 56 597 1963.

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Clinical and Serological Observations in Cases of Coxsackie B3 Infections in Early Infancy

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This report presents seven patients with Coxsackie B3 infection in early infancy The clinical picture suggested several diagnostic possibilities until the results of virological and serological investigations indicated the etiology The preliminary virological information obtained allowed isolation measure to be taken to prevent further spread of the infection.

The main purpose of the paper however is to illustrate a simple way of differentiating antibodies of maternal and infantile origin. As is well known the interpretation of serological findings in early infancy may be particularly difficult since the antibody titer found in the serum of an infant can be partly or totally derived from passively transferred maternal antibodies [12]. These maternal antibodies are γG-globulins (7S γ-globulins) whereas the early antibodies formed by the infant in response to an infection are γM globulins (19S γ-globulins) [3 7 10]. They can be easily differentiated because the γM globulins usually lose their antibody activity after treatment with reducing agents such as 2 mercaptoethanol, whereas the γG-antibodies do not [1 6, 9]. We have found this technique most helpful in estimating the infants own antibody response thus facilitating the interpretation of serological results during this period of life

Material and Methods

The patients presented in Table 1 are all from the Gothenburg area.

Isolation of virus Faecal specimens, nose and throat swabs and cerebrospinal fluid (CSF) were used for the isolation of virus. From one fatal case brain, lung and liver specimens were obtained at autopsy These latter specimens, ground with a mortar in sterile sand, were suspended in Hanks balanced salt solution. After centrifugation the supernatant was inoculated into cell cultures.

Each specimen was added to two monkey cell cultures and to two cultures of HeLa cells. The cells were maintained in Hanks balanced salt solution with 0.5% lactalbumin hydrolysate, 7% calf serum and antibiotics. The cultures were examined daily for 12 days.

Neutralization tests. These tests were performed with HeLa cell cultures using 100 $TCID_{50}$ of a reference Coxsackie B3 virus and serial two-fold dilutions of the serum to be tested The reference virus and the

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serum dilution were mixed and allowed to stand at room temperature for one hour and the mixture was thereafter inoculated into three cultures.

Reduction with Mercaptoethanol. The reduction was performed essentially according to Schrohenloher *et al.* [9]. The sera were dialysed for 3 hours at room temperature against phosphate buffered saline containing 0.2 M 2 mercaptoethanol. Thereafter dialysis was performed for 4 hours against the same buffer containing 0.02 M iodacetamide in order to alkylat the reduced antibodies. Finally the sera were dialysed overnight in the cold gainst the buffer only

Findings and Comments

The pertinent clinical data are given in Table I

Virus isolation Coxsackie virus, type B3 was isolated from the faecal specimens of six of the patients. In the remaining case (Case 1 Table 1) which was fatal, the virus was isolated from brain, liver and lung specimens taken at the autopsy In addition, Coxsackie B3 virus was recovered from nasal secretions in two cases and from CSF in one case. These findings, and the serological observations, indicated a common etiology in the seven cases, although the clinical picture varied considerably (Table 1)

Isolation of a virus and a rise in the titer of specific antibodies to that virus from the acute phase of the disease to the convalescent phase are generally accepted criteria for the existence of a causal relationship between the virus isolated and the clinical disorder However these criteria fulfilled, there still exists the possibility that a clinically silent viral infection may appear concomitantly with a disease of another etiology thus causing misinterpretation. This must always be borne in

TABLE 1 *Clinical and laboratory findings in seven patients with Coxsackie B3 infections*

Case No. and birth weight	Age at onset of sympt.	Symptoms on admission	Course and follow up (time after admission)	Epidemiology	Cerebrospinal fluid and blood findings on admission: Total white cells per mm³	Poly per cent	Mono. per cent	Protein 100 ml	Max. Micro sed. rate mm/hour
1 A 63.07 27	*One hour after birth*	*Temp. 38.* Resp. distress. Grey colour Irritability Increased tone	*Gradually worse.* Died on 3rd day Autopsy: inflammatory reactions in CNS	Mother had upper resp inf 7 10 days before delivery	*CSF 100*	20	*80*	—	*ND*
1000 g					Blood ND	—	—		
2. B. K. 63.07 _3	7–8 days	N fever Spells of pnoea. Moderate icterus. Lethargy	Frequent attacks of apnoea. Artificial respiration 3 days. Rapid	Shared room with Case 1 on second and third days of lf	CSF 486	23	77	—	41
					CSF next } (203	58	42	49)	

[illegible] 65.09.16	[illegible]eek	Temp. [illegible] Good general condition	[illegible] A[illegible] the age of 13 day respiratory arrest. Artificial respiration 2 days. Then rapid recovery 6 weeks healthy	—	NP 250	[illegible]		[illegible]	
3050 g					Blood 14200	73	25		
4 R. G. 65.06.03	5 [illegible]eeks	Temp. 39 Sluggish	Diphasic febrile course. Afebrile few hours after admission. N[illegible] other symptoms. 8 months healthy	Parents and brother had fever headache and myalgia simult. with patient. Mother had Cox. B3 infection.	CSF 81	—	—	—	41
4710 g					Blood 8200	22	78		
5. M. M. 65.07.06	8 [illegible]eeks	Temp. 39 Sluggish, pale. Whining. Slightly bulging fontanel	Afebrile after 3–4 days. Rapid recovery 3 months healthy	Mother hospital. sad same day because of fever stiffness of neck	CSF 536	76	24	91	28
3630 g					Blood 17000	48	52		
6 C. R. 65.03.31	6 months	Temp. less than 38. Generalized convulsions. Unconscious. Increased musc. tone. Deviation of eyes t[illegible] the left. Petechiae on the legs	Vomiting, fever for one week. Successively brighter during this period. N[illegible] convulsions. Paresis of ocular muscles? for 3 days. 10 [illegible]eeks healthy	10-year-old brother fell ill with upper resp. inf. fever headache one week before pat.	CSF 1	—	—	26	21
18[illegible] g					Blood 4800	40	60		
7 R. R. 65.03.31	6 months	Temp. 37.8. Generalized convulsions. Vomiting. Sluggish. Petechiae on the legs	Same as sibling (Case 6) but no paresis of ocular muscles. 10 weeks healthy	Sibling Case 6	CSF 32[a]	40	60	74	20
2250 g					Blood 9100	50	50		

[a] 40,000 red cells per mm

mind in discussions of the association between clinical data and virological findings.

Clinical observations In three patients who fell ill during the newborn period (Cases 1–3) respiratory symptoms dominated the clinical picture. In one case there was a respiratory distress syndrome in the two others there were spells of apnoea which necessitated tracheotomy or intubation and artificial respiration for a few days. These symptoms led to our performing lumbar puncture which showed pleocytosis, suggesting a cerebral origin of the respiratory difficulties These three cases, of which one died, emphasize the risk of severe disease due to Coxsackie B infections in the neonatal period.

In the other somewhat older patients, other cerebral symptoms, such as sluggishness or convulsions with unconciousness dominated the clinical picture Only one of the patients had a definitely bulging fontanel Nuchal rigidity or a Brudzinski sign was not present in any case. EEG was normal in cases 2 and 5 four and seven weeks after onset of their disease. No EEG studies were done during the acute phase of the disorder

Disregarding the pulmonary syndromes, which probably were of central origin, in the three newborns there were no marked clinical signs of upper or lower respiratory tract infection. Nor were there any gastrointestinal symptoms. ECG was not investigated during the acute phase of the disease. However there were no clinical signs of myocarditis.

Laboratory findings As many as 300–500 cells were observed in CSF of three of the patients. Mononuclear cells usually dominated, but polymorphonuclear leukocytosis was also observed (Case 5) in the CSF It is of interest that although there was pleocytosis in the CSF of five patients two patients with clinically severe cerebral symptoms showed no pathological findings in the CSF at the only examination performed. This may suggest that in these cases the inflammatory reaction of the central nervous system was not localized to the meninges.

The sedimentation rate was elevated in 5 of 6 patients including one neonate. A rather high white blood cell count was observed in 3 of 6 patients with a polymorphonuclear cell dominance in one of them. These findings indicate that little is gained by such laboratory investigations when attempting to differentiate between infections of viral or bacterial origin in small infants. Bacterial cultures from CSF and other locations did not yield any pathogens.

Epidemiology These cases all appeared during a short period of time July–September 1966 when an outbreak of Coxsackie B3 infection occurred in the Gothenburg area. As demonstrated by Table 1 both family infections and nosocomial transmission seemed to be responsible for the infection of the infants.

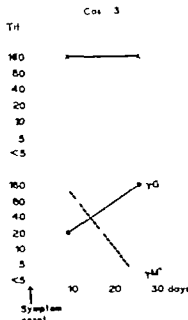
The fatal case (Case 1) showed symptom of disease one hour after birth. The mother of this infant, who died on the third day of life had had an upper respiratory infection 7 to 10 days before delivery Histological examination of the infant demonstrated inflammatory reaction in various organs, particularly the brain and was thus suggestive of an intrauterine transmission of virus (Kristensson & Sourander to be published) There are only a few cases reported where intrauterine

infections with Coxsackie B viruses have been observed [5]. As in this case, the *mothers of such infants seem to have been* infected shortly before delivery and the infection in the newborn was often fatal.

In all the cases virus was demonstrated within three days after the specimens were obtained. This allowed measures to be taken preventing spread of the infection in the ward. All infected infants were isolated and faecal samples from other patients and all personnel involved were examined. No virus isolations were obtained from these samples.

Serological observations Serological studies can provide supportive evidence for the presence of a virus infection. During the first few months of life antibody titers demonstrable in the infant's serum cannot however be directly accepted as an indication of infection in the infant, as the titre may be due to passively transferred maternal antibodies [3, 13]

The early antibody response of the neonate as well as that of the adult is made up of γM-globulins [3, 6, 7 10], these, however do not cross the placenta. γG-antibody production follows γ-M antibody production in neonates as well as in adults. Thus γM-antibodies in the serum of the neonate must be of infantile origin, whereas the γG-antibodies may originate either from the infant or from the mother When only γG ant bodies are present in the newborn it is usually presumed that



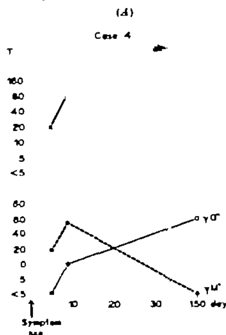


Fig. 1 (*A*) Case 3. The total ant body titre is equal on two examinations (upper diagram). When the titre is divided into γM and γG-antibodies rather dramatic changes are uncovered (lower diagram): the infant initial γM antibodies decrease, while the γG antibodies increase. ×—× Total antibody titre; O—O γG titre; ● ● γM-titre (calculated as difference between total titre and γG-titre). (*B*) Case 4. Also in this patient it is well illustrated how the initial γM response is taken over by γG-response. The total titre show only small changes. All antibodies are *of the infant* own production. Symbols as in Fig. 1 (*A*).

TABLE 2 *Serological findings in seven cases of Coxsackie B3 infections*

Case	Age of patient	Duration of disease	NT[a] titre of untreated serum	NT-titre of mercaptoethanol treated serum	Type of antibody[b] γM	γG
1	2 days	2 days (congenital)	5	5	–	+
2	13 days	7 days	3	5	–	+
	27 days	21 days	320	80	+	–
	39 days	33 days	160	160	–	+
3	14 days	8 days	160	20	+	+
	3_ days	28 days	160	160	–	+
4	36 days	3 days	20	< 5	+	
	40 days	9 days	80	10	+	+
	8 months	5 months	60	80	–	+
5	2½ months	7 days	160	< 5	+	
	3 months	_3 days	640	160	+	+
6	6 months	2 days	< 5	< 3		
	8½ months	2½ months	> 1280	> 1280	–	+
7	6 months	2 days	< 5	< 5		–
	8½ months	2½ months	> 1280	> 1280		+

[a] NT = neutralization test.
[b] + and – indicates presence or absence of antibody type.

they originate from the mother and are passively transferred.

These two antibody types can be differentiated by reduction with mercaptoethanol (2-ME), because the γM antibodies lose their antibody activity after reduction whereas the γG-antibodies do not. A few exceptions to this have been observed [9]. The place of γA globulins in the antibody response is not well known but they are also reported to usually be sensitive to reducing agents [7]. Aware of the fact that reduction of antibodies does not definitely characterize them, we have used the simplification of calling the 2-ME-sensitive ones 19S or γM globulins, and the 2-ME resistant ones for 7S or γG globulins.

In the single sample obtained before death the first case had only a low titre of ME-resistant antibodies, i.e. most probably γG-antibodies originating from the mother (Table 2). In the second case a low titre (five) of γG-globulin antibodies was found. These antibodies could have been passively transferred. However in the next sample a much higher titre of 80 of γG-antibodies was found together with some γM-antibodies. Both of these antibodies must have been formed by the infant and when the second sample was obtained the primary γM-antibody response was probably just being taken over by the γG-antibody response, which experimentally has been shown to inhibit further production of γM-antibodies [8]. In the last sample from this child one month after the onset of the disease, only γG antibodies were found in a titre of 160. In Cases 3–5 γM-antibodies were found in the early blood samples, but in the later samples the infant had gone over to production of more and more γG-antibodies. This is graphically illustrated in Figs. 1 (*A*) and (*B*). Hence it was possible to establish the child's own antibody response to viral infection as early as the first blood sample in these 3 cases. These findings are consistent with the pattern of antibody response seen in humans after stimulation with viral and bacterial antigens [3, 6, 12], as well as to immunization of rabbits with poliovirus [11]. In the 6-month-old twins (Cases 6 and 7) no antibodies were found in blood samples taken after two days of illness, but ten weeks later they both had a very high titre of γG-antibodies of their own production (Table 2).

The γG-antibody response followed upon the γM response in some cases (Nos.

–4) as early as 1–3 weeks after the clinical onset of infection. This early appearance of γG-antibodies in an immature infant is a finding which contrasts, to some extent, with the results of immunization with e.g. salmonella H-antigen [10]. The antibody response of the newborn to these antigens was found to be of the γM type for as long as two months following immunization. Uhr *et al.* [12] however reported that premature infants responded to injection with bacteriophage ΦX 174 by initially forming antibodies of the 19S type and then, 2–6 weeks after the immunization, by formation of antibodies of the 7S type. Even before birth of the infant γM-antibody production has been demonstrated in response to viral infections [2]. Van Furth *et al.* have shown small amounts of γM globulins in cells in spleen and circulation from fetuses 20 weeks and older; in a few instances γG globulins were also observed [4].

Summary

Clinical and virological findings in seven children diseased during early infancy are reported. Serological observations and virus isolations indicated a common etiology (Coxsackie B3 virus) although the clinical picture varied. In the neonatal period respiratory symptoms, most probably of central origin, dominated, whereas in older infants other cerebral symptoms, such as sluggishness or convulsions with unconciousness, were dominant. The diagnostic usefulness of treatment of serum samples with – mercaptoethanol for the differentiation between maternally transferred ant bodies and antibodies formed by the infant itself is illustrated and emphasized.

References

1. DEUTSCH, H. F. and MORTON, J. J.: Dissociation of human serum macroglobulins. *Science, 125:* 600, 1957.
2. ECHENWALD, H. F.: cited by GOOD, R. A. and PAPERMASTER in *Advances Immunol 4:* 26, 1964.
3. FINK, C. W., MILLER, W. E., DORWARD, BARBARA and LOSPALLUTO, J.: The formation of macroglobulin antibodies. II. Studies on neonatal infants and older children. *J Clin Invest, 41:* 1422, 1962.
4. FURTH, R. VAN, SCHUIT, HENRICA, R. E. and HIJMANS, W.: The immunological development of the human fetus. *J Exp Med, 122:* 1173, 1965.
5. KIBRICK, S.: Current status of Coxsacki and ECHO viruses in human disease. *Progr Med Virol, 6:* 27, 1964.
6. LOSPALLUTO, J., MILLER, W., DORWARD, BARBARA and FINK, C. W.: The formation of macroglobuli antibodies. I. Studies on adult humans. *J Cl I vest 41:* 1415, 1962.
7. MANNIK, M. and KUNKEL, H. G.: The immunoglobulins in M. Samter (ed.): *Immunological Diseases.* Little Brown, London, 1965.
8. MÖLLER, G. and WIGZELL, H.: Ant body synthesis at the cellular level. Antibody induced suppression of 19S and 7S antibody response. *J Exp Med 121:* 969, 1965.
9. SCHROHENLOHER, R. E., KUNKEL, H. G. and T MASI, T. B.: Activity of dissociated and reassociated 19S anti γ-globulins. *J Exp Med, 120:* 1215, 1964.
10. SMITH, R. T.: Response t active immunization of human infants during neonatal period, in G. E. W. Wolstenholme and M. O Connor (ed): *Ciba Foundation Symposium on Cellular A pects of Immunity.* Little Brown, Boston, 1960.
11. SVEHAG, S. E. and MANDEL, B.: The formation and properties of polio irus neutralizing antibody. I 19S and 7S antibody formation: differences in kinetics and antigen dose requirement for induction. *J Exp Med, 119:* 1, 1964.
12. UHR, J. W., DANCIS, J., FRANKLIN, E. C., FINKELSTEIN, M. S. and LEWIS, E. W.: The antibody response to bacteriophage ΦX 174 in newborn premature infants. *J Clin I vest 41:* 1406, 1962.
13. VAHLQUIST, B.: Transfer of antibodies from mother to offspring. *Advances Pediat 10:* 305, 1958.

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Haptoglobin Level in Some Diseases of Infancy and Childhood

by M KAMAL BADR EL-DIN MOSTAFA KHALIL and A. SAMIR KASSEM

It is well established that the level of haptoglobin can be taken as an index of increased erythrocyte destruction [1 8, 16] Plasma haptoglobin decreases in haemolytic states as it rapidly combines with haemoglobin and the resulting haemoglobin–haptoglobin complex is within hours removed from the circulation by the reticulo-endothelial system [9] Further studies have also shown that haptoglobin behaves as an acute phase reactant and some tuberculosis centres in France use its estimation as a routine laboratory aid to assess the extent and activity of the tuberculous lesion [5 1 6, 15]

The aim of the present study was to further evaluate the changes in haptoglobin level during the course of some haemolytic disorders as well as in other common diseases of infancy and childhood. Rheumatic fever acute nephritis, nephrotic syndrome and virus hepatitis were accordingly also investigated.

Method and Technique

Haptoglobin estimation was done by the activation method of Jayle [4]. The results are expressed as haemoglobin binding capacity (HbBC) in milligrams per cent i.e. the amount of haemoglobin in milligrams which can be bound by the haptoglobin present in 100 ml of a solution.

Material and Results

Normal controls

A control study was made on 187 normal infants and children from birth up to the age of twelve years. The mean HbBC was 0 at birth and then progressively increased to reach the stabilised level of 100.3 mg/100 ml serum ±29.3 after the age of six months.

Favism

Serial estimation of haemoglobin and haptoglobin levels was carried out on six infants and children (Table 1) The onset of haemolysis before admission was from one to seven days. Every case received a blood transfusion on admission and no further treatment was thereafter instituted.

The total group had on admission a mean HbBC of 10 7 mg/100 ml serum ±18.3 and within three days of the onset of haemolysis all cases showed ahaptoglobinaemia The follow up study revealed progressive increase in haemoglobin concentration and HbBC A measurable amount of haptoglobin started to appear in the serum on the fourth day after the onset of haemolysis and gradually increased until it reached the normal level 5–6 days afterwards.

TABLE 1. *Values of haemoglobin (g/100 ml) and HbBC (mg/100 ml serum) in cases of favism*

No.	Age (years and months)	Duration before admission (days)	Day of admission Hb	Day of admission HbBC	3rd day Hb	3rd day HbBC	5th day Hb	5th day HbBC	7th day Hb	7th day HbBC	9th day Hb	9th day HbBC	11th day Hb	11th day HbBC
1	4 y	2	2.5	0	7.8	10	10	41	10.8	82	11	103	11.5	105
2	3 y	3	6.5	0	8	31	9.5	65	10.5	91	10.3	106	11	104
3	3 m.	5	3.5	0	7	23	8	49	10.5	0	11	73	11.3	72
4	8 m.	5	3	12	6	40	6.5	69	8	93	8.5	90	9	93
5	10 m.	7	2.3	45	6.7	72	9	92	10.5	98	11	95	12	98
6	18 m.	7	3.8	20	6.5	0	7	88	7.5	124	8	124	9	131

Thalassemia Major

Seven children with Thalassemia Major and ranging in age from one to 13.5 years were investigated. They were kept for one month under observation without any specific treatment and then a blood transfusion was given once weekly for a period of another month. Thereafter three children were kept on dexamethasone therapy and two were splenectomized. Serial estimation of haemoglobin and haptoglobin levels was done every week.

As is shown in Table 2, all cases had ahaptoglobinaemia on admission and during the one month observation period. Repeated blood transfusions did not affect either the haemoglobin or haptoglobin levels. Only one of the three children on dexamethasone showed improvement in haemoglobin concentration. However the HbBC was consistently zero in the three of them. The two children submitted to splenectomy showed significant elevation in haemoglobin concentration. In one of them, there was a transient rise in HbBC. This rise coincided with wound infection and the level rapidly returned to zero when the infection subsided.

TABLE 2. *Values of haemoglobin (g/100 ml) and HbBC (mg/100 ml serum) in cases of Thalassemia Major and different lines of treatment*

Case No.	Values on admission Hb	Values on admission HbBC	Values at end of observation period Hb	Values at end of observation period HbBC	Values after blood transfusion Hb	Values after blood transfusion HbBC	Values after dexamethasone Hb	Values after dexamethasone HbBC	Values after splenectomy Hb	Values after splenectomy HbBC
1	9	0	9	0	10.5	0	11.8	0		
2	4	0	4	0	4.5	0			10.3	99 (during infection) then 0
3	3.5	0	3.6	0	4	0			8.5	0
4	7.5	0	6.5	0	8.3	0	7.3	0		
5	9	0	8.8	0	10.8	0				
6	4	0	4	0	4.3	0	4.5	0		
7	8.8	0	6.5	0	8	0				

TABLE 3 *Results of serial estimation of HbBC in two cases of Thalassemia Major after intramuscular milk injection.*

Case No.	HbBC (mg/100 ml serum) after milk injection				
	12 hours	24 hours	36 hours	48 hours	96 hours
1	0	30	52	80	45
2	0	70	50	60	35

Infection as a form of stress was thought possibly to be the factor responsible for the transient rise of haptoglobin in this case. To study this effect, milk injection was given to two thalassemics and the haptoglobin level was estimated thereafter (Table 3). A measurable haptoglobin level was detected 24 hours after milk injection reached a maximum after 48 hours and then gradually dropped.

Acute rheumatic fever

This study included twelve children ranging in age from 6 to 14 years four males and eight females.

All twelve patients showed evidence of carditis and four of them had in addition congestive heart failure. Therapy included penicillin, aspirin and/or cortisone. Children with heart failure also received digoxin and diuretics. One child died soon after admission. Serial estimations of HbBC and ESR were done in all cases.

On admission, the mean HbBC in the eight cases with no heart failure was 327 mg/100 ml serum ±61.2 (Table 4). This is significantly higher than that for normal controls. The ESR was high in all cases. It ranged between 40 and 120 in the first hour and between 88 and 141 in the second hour.

The mean HbBC in the four cases with congestive heart failure was on admission 86.8 mg/100 ml serum ±43.4 (Table 5). This is not significantly different from that of normal controls. The ESR varied between 7 and 40 in the first hour and 22 and 60 in the second hour.

TABLE 4 *Behaviour of HbBC (mg/100 ml serum) and first hour sedimentation rate in cases of rheumatic carditis with no heart failure*

Case No.	On admission		After 10 days		After 20 days		After 30 days		After 40 days	
	HbBC	ESR	HbBC	ESR	HbBC	ESR	HbBC	ESR	HbBC	ESR
1	340	73	264	40	173	20	120	13	97	5
2	384	84	158	5	198	13	160	8		
3	190	40	126	13	124	5	122	3		
4	300	70	246	60	229	30	188	14	130	8
5	362	129	232	13	217	10	144	2	140	2
6	260	90	194	20	172	15	136	9		
7	220	90	223	20	163	15	150	3		
8	240	100	232	30	180	15	157	6	142	6

TABLE 5 *Behaviour of HbBC (mg/100 ml serum) and first hour sedimentation rate in cases of rheumatic carditis with heart failure*

Case No.	On admission		After 10 days		After 20 days		After 30 days		After 40 days	
	HbBC	ESR	HbBC	ESR	HbBC	ESR	HbBC	ESR	HbBC	ESR
1	74	7	124	8	163	21	142	1	120	10
2	87	40	173	40	142	20	107	10		
3	41	12	107	10	103	8				
4	145	18								

In cases with no heart failure the ESR returned to normal after a period of 20 to 40 days. Normal values of HbBC were however reached after 10 to 40 days

Acute glomerulonephritis

Twenty children were included in this study Their age ranged between – and 7 years and the duration of illness before admission was between three days and one month. Congestive heart failure was present in five cases. A follow up study with estimation of HbBC and ESR every ten days was carried out on six cases. Tables 6 and 7 show the results obtained.

Results of the follow up study show that the HbBC dropped in a smooth fashion that was parallel to clinical and laboratory improvement. The ESR however lagged in three cases behind the HbBC

Nephrotic syndrome

Fourteen children were investigated. Their age ranged between 1.5 and 11 years, and the duration of their complaint was between four days and two months Table 8 shows the mean, range and standard deviation of HbBC and the mean and range of ESR.

Five cases were followed up and were treated with penicillin and dexamethasone It is evident (Table 9) that two cases showed return of ESR to normal, while the HbBC was still high At that time oedema and/or albuminuria were still present. One case showed return of HbBC to normal while the ESR was still high. At that time, oedema and albuminuria had disappeared.

Virus hepatitis

Seventeen cases of virus hepatitis with an age ranging between 10 months and

TABLE 6. *HbBC and first hour sedimentation rate in acute nephritis*

Acute nephritis	No. of cases	HbBC (mg/100 ml serum) Mean	Range	Standard deviation	First hour ESR Mean	Range
With no heart failure	15	232 4	113–436	78.8	48.6	10–110
With heart failure	5	230.2	182–302	58.3	40.4	10– 93
Total	20	231 9	113–436	72.2	46.5	10–110

TABLE 7 *Follow-up values of HbBC (mg/ 100 ml serum) and first hour sedimentation rate in acute nephritis*

No. of cases		On admission	After 10 days	After 20 days	After 30 day	After 40 days	After 50 days
1	HbBC	258	195	186	183	132	111
	ESR	93	125	90	58	25	15
	Albuminuria	+	+	+		−	−
	Haematuria	+	+	+	−	−	−
2	HbBC	214	180	142	144	140	128
	ESR	85	40	15	20	17	17
	Albuminuria	+	+	−	−	−	−
	Haematuria	+	+	−	−	−	−
3	HbBC	290	227	186	173	161	149
	ESR	100	90	60	35	33	25
	Albuminuria	+	+	+	−	−	−
	Haematuria	+	+	+	−	+	−
4	HbBC	286	258	237	207		
	ESR	70	80	60	40		
	Albuminuria	+	+	+	+		
	Haematuria	+	+	+	−		
5	HbBC	436	198	132	153	120	
	ESR	30	20	5	3	2	
	Albuminuria	+	+	−	−	−	
	Haematuria	+	−	−	−	−	
6	HbBC	200	14	172	133		
	ESR	35	14	10	3		
	Albuminuria	+	−		−		
	Haematuria	+	−	−	−		

10 years were studied. The duration of the disease varied between seven days and one year. One case showed clinical manifestations of precholemia and another had manifest cholemia. Oedema and massive ascites were present in one case with chronic hepatitis of one year duration.

The total group had a mean HbBC of 42.5 mg/100 ml serum ±46.6. This is significantly lower than that for normal controls. There was no parallel correlation between the HbBC and either the level of plasma proteins or thymol turbidity. However there was a good correlation between the level of haptoglobin and the chronicity of the disease (Table 10).

A follow up study was made on four cases in which the duration of jaundice

TABLE 8. *HbBC and sedimentation rate in nephrotic syndrome.*

No. of cases	HbBC			ESR (1st hour)		ESR (2nd hour)	
	Mean	Range	Standard deviation	Mean	Range	Mean	Range
14	237.1	204–277	43.9	94.6	40–140	112.7	85–100

Table 9 *Follow up values of HbBC (mg/100 ml serum) and first hour sedimentation rate in nephrotic syndrome*

Case No.		On admission	After 10 days	After [illegible]	After 30 day
1	HbBC	373	284	[illegible]	84
	ESR	125	100	[illegible]	45
	Oedema	+ +	+		−
	Albuminuria	+ + +	−		−
2	HbBC	360	263	4	124
	ESR	118	43	18	5
	Oedema	+ +	−		−
	Albuminuria	+ + +	+		−
3	HbBC	318	1 5	16	140
	ESR	40		3	3
	Oedema	+ +	−		
	Albuminuria	+ +	+		−
4	HbBC	372	318	233	13
	ESR	117	10	5	3
	Oedema	+ + +	−		
	Albuminuria	+ +			
5	HbBC	272	236	110	8
	ESR	82	50	8	2
	Oedema	+ + +	−	−	
	Albuminuria	+ +	−	−	−

was 20 days or less. The HbBC was found to return to normal values after a period of 16 days to 3 months from the onset This coincided with clinical recovery and the return to normal of the liver function tests.

Discussion

Haptoglobin level, being an index of increased erythrocyte destruction, has been used in the present study to evaluate various measures in the treatment of haemolytic anaemias

Table 10 *Relation of HbBC to chronicity of virus hepatitis*

No. of cases	Duration of disease	HbBC (mg/100 ml serum) Mean	Range
8	7 days or less	78 3	6–150
4	8 days–1 month	19	0– 45
3	1 month–4 months	6.7	0– 20
2	1 year	0	0

Six cases of favism were studied. The only therapeutic measure taken in all cases was a single blood transfusion given on the day of admission. Serial determination of haptoglobin revealed its absence in all cases during the first three days after the onset of haemolysis Thereafter a measurable amount could be detected and normal levels were attained 9–11 days from the onset Assuming that the usual duration of haemolysis in favism is –3 days [10], it can be suggested that regeneration of haptoglobin to normal required 6–8 days which is in agreement with the findings of Kander & Mauer [7] who reported that the level of haptoglobin remained below normal for 5–7 days after the stoppage of haemolysis

The second haemolytic disorder studied was Thalassemia Major Results of the follow up study when no treatment was given showed that the haemoglobin level did not show any significant change and the haptoglobin level was consistently zero denoting continuous destruction of red blood corpuscles and continuous utilization of haptoglobin.

The effect of repeated blood transfusions, cortisone administration and splenectomy were evaluated. There was persistent ahaptoglobinaemia during the transfusion therapy The haemolytic process was further found to involve the transfused cells as there was no concomitant rise in the haemoglobin level.

Serial determination of haemoglobin and haptoglobin levels in three cases given dexamethasone for one month revealed a significant rise in haemoglobin in one case and almost no change in the other two The haptoglobin level was persistently zero in all cases suggesting that the benefit occasionally seen from dexamethasone therapy is probably through stimulation of the bone marrow to produce more cells rather than inhibiting red cell destruction.

In the two cases submitted to splenectomy a significant rise in haemoglobin level was achieved The HbBC was persistently zero in one case. In the other case, there was a transient significant rise one week after operation This rise coincided with the development of wound infection and when this subsided the level of haptoglobin returned to zero The effect of stress on the level of haptoglobin was studied by the injection of milk and it was verified that it is a factor responsible for the rise of haptoglobin level. These data strongly suggest that in Thalassemia Major ahaptoglobinaemia is not due to exhaustion and/or inability of the tissues to form haptoglobin but due to increased consumption by the continuously released haemoglobin.

A correlation between the haptoglobin level and the ESR was made in cases of acute rheumatic fever The results of this study revealed significant elevation of both haptoglobin and ESR in those cases presenting with active carditis but no heart failure. Those presenting with heart failure, on the other hand, revealed either low or normal levels of haptoglobin and normal or moderately elevated ESR. When the heart failure was controlled, both haptoglobin and ESR increased to normal or higher levels according to the state of activity and thereafter they smoothly declined with clinical improvement.

In the follow up study haptoglobin level appeared to express closely the degree of inflammatory activity It showed a smooth and consistent decline with decrease in activity thus providing clear separation between active and inactive phases of the disease Being a glycoprotein in nature haptoglobin could be related to the serum glycoprotein previously studied by Shetlar *et al* [14]

The mean HbBC in acute glomerulonephritis was significantly higher than the normal value. Similar results were reported by Jayle [3] The level was found to return to normal with complete clinical and laboratory cure The ESR in this series was not elevated in all cases during the active phase and in some cases its return to normal lagged after clinical and laboratory cure

In cases of the nephrotic syndrome, the haptoglobin level was always significantly high during the active phase. These high

values could be related to the high level of alpha globulins previously reported [11] Results of the follow up study suggest that haptoglobin estimation in all cases may reflect closely the state of activity better than the ESR.

In cases of virus hepatitis, the level of haptoglobin was significantly lower than normal. This diminution had no relation either to the plasma protein level or to the thymol turbidity or icterus index. Nyman [15] also found subnormal haptoglobin levels and transient ahaptoglobinaemia in acute hepatitis and toxic hepatocellular disease. In the present study there was a good correlation between chronicity of the disease and the diminution in haptoglobin level. This agrees with the findings of Hever & Vadasz [2] Cases presenting with jaundice and active hepatitis of one week or less showed a mean HbBC of 78.3 mg/100 ml serum. Those presenting with active hepatitis of eight days to one month, had a mean value of 19 mg/100 ml serum, and those in which the duration was between one and four months had a mean value of 6.7 mg/100 ml serum In two cases with chronic hepatitis of one year duration, the haptoglobin was zero. It may be concluded that serial determination of haptoglobin in cases of virus hepatitis is highly significant in the evaluation of the disease process and could be taken as a prognostic test. Cases going to chronicity showed marked lowering while those proceeding to recovery showed a progressive rise in haptoglobin level.

Summary

The level of serum haptoglobin was estimated in favism thalassemia major acute rheumatic fever acute glomerulonephritis, nephrotic syndrome and virus hepatitis.

In favism, it was totally absent during the haemolytic episode and then started to rise till it reached normal values after 6–8 days.

Ahaptoglobinaemia was persistent in patients with Thalassemia Major before and after treatment with blood transfusion, dexamethasone administration and splenectomy

In acute rheumatic fever acute glomerulonephritis and nephrotic syndrome, there was elevation of the haptoglobin level. This could therefore be taken as one of the criteria to evaluate activity of the disease process.

The level was low in virus hepatitis and returned to normal with cure There was a good correlation between the chronicity of the case and the diminution in haptoglobin level.

References

1 Allison, A. C. The genetical and clinical significance of the haptoglobins. *Proc Roy Soc Med*, *51* 641 1958.

2. Hever, O. and Vadasz, O Haptoglobin in children with infectious hepatitis. *J Pediat*, 67 1136, 1965.

3. Jayle, M. F Les modifications biochimiques et physicochimiques du plasma sanguin au cours de l'infection. *Expos Ann Biochim Med* 3 162, 1942.

4 — Méthode de dosage de l'haptoglobine sérique *Bull Soc Chim Biol*, *33* 8 6, 1951

5. Jayle, M. F and Boussier, G Les séromucoïdes du sang, leurs relations avec les mucoprotéines de la substance fondamentale du tissu conjonctif *Expos Ann Biochim Med*, *17* 15 1955

6. Jayle, M F and Coxas, G Propriétés et constitution chimique de l'haptoglobine sérique. *Bull. Soc Chim. Biol* *34* 83, 1952.

7. KAUDER, E. and MAUER, A. M.: The physiology and clinical significance of haptoglobin. *J Pediat, 59*: 286, 1961
8. LATHEM, W. and JENSEN, W.: Plasma haptoglobin-binding-capacity in sickle cell disease. *Blood, 14* 1047, 1959.
9. MURRAY, R. K., CONNELL, G. E. and PERT, J. H.: The role of haptoglobin in the clearance and distribution of extracorpuscular haemoglobin. *Blood, 17* 45, 1961
10. NELSON, W. *Textbook of Pediatrics* Saunders, Philadelphia and London, 1964, p. 1022. 8th edition.
11. — *Textbook of Pediatrics*. Saunders, Philadelphia and London, 1964, p. 1128. 8th edition.
12. NOSSLIN, B. F. and NYMAN, M.: Haptoglobin determination in diagnosis of haemolytic disease. *Lancet, 1* 1000, 1958.
13. NYMAN, M.: Serum haptoglobin, methodological and clinical studies. *Scand. J Clin Lab Invest Suppl. 39* 1959
14. SHETLAR, M. R., PAYNE, R. W., STIDWORK, H. B. and FAULKNER, J. B. Objective evaluation of patients with rheumatic diseases. Comparison of serum glycoprotein, seromucoid and c-reactive protein determination as methods for the evaluation of patients with rheumatic fever. *J Pediat 51* 810, 1957
15. TROISIER, J., POLONOVSKI, M., JAYLE, M. F and BRISSAUD, H. E.: Indice de paraptoglobinemie chez les tuberculeux. *Bull Acad Med, 1.3.* 239, 1940
16. WHITTEN, C. F.: Studies on serum haptoglobin. A functional inquiry. *New Engl J Med, 266.* 529, 1962.

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Arterial Blood Gas and Acid Base Balance in the Newborn Infant Effects of Cord Clamping at Birth

by WILLIAM OH [1] RENE A. ARCILLA,[2] JOHN LIND and IRA H GESSNER

Recent studies have shown that the amount of placental blood transfusion to the newborn infant at the time of its birth is readily altered by early or late clamping of the cord, and that this may influence some of the physiologic changes that normally transpire during the early hours of life. Infants with early cord clamping tend to have lower respiratory rates [8], higher lung compliance associated with a greater functional residual capacity [9] than the late clamped subjects during the first few hours of life. In addition, the former have significantly lower pulmonary arterial pressures [1] and possibly lower pulmonary vascular resistance [3] than the latter during this same period. These differences could conceivably alter the nature of gaseous exchange in the lung

The present communication deals with a comparative study of the arterial blood gas and acid base status of infants delivered with early and with late clamping of the cord, during the first 24 hours of life This study was done during the course of our hemodynamic studies on these two groups of subjects [1 –].

Material and Methods

The subjects of this study were 118 normal term newborn infants born vaginally to healthy mothers after 37 to 43 weeks of uneventful pregnancy Duration of labor ranged from 2 to 48 hours; 41 mothers had short intermittent inhalation of nitrous oxide ½ to 3 hours before the delivery and 20 mothers had intramuscular Pethidine 50 to 100 mg 2 to 12 hours before the delivery All infants were delivered in cephalic presentation, without forceps application. All were in good condition at birth; none required resuscitation or oxygen therapy

The infants were divided into two groups:

1 Early clamped group (37 infants) cord clamped within 5 to 10 seconds after delivery of the buttock

2. Late clamped group (81 infants) cord clamped after cessation of arterial pulsation.

The infants were delivered int bed situated at the foot of the delivery table

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Supported by the Advanced Research Fellowship of the American Heart Association and the Heart Association of Palm Beach and Martin Counties (Florida). Assistant Professor of Pediatrics, University of Florida, Gainesville, Florida.

TABLE 1 *Birth weight analgesia anesthesia duration of labor and body temperature of 118 term newborn infants with early and late cord clamping*

	Late clamped group	Early clamped group
Birth weight (grams)		
Mean	3490	3330
Range	([illegible]850–4550)	([illegible]680–4400)
Anesthesia during labor[a]	27 mothers	14 mothers
Analgesia during labor[b]	14 mothers	6 mothers
Duration of labor (hours)		
Mean	6.5	10.6
±S.E.M.	±0.7	±1.9
Body temperatures (°C)		
Mean	35.9	35.7
±S.E.M.	±0.1	±0.09
Total no. of infants	81	37

Intermittent nitrous oxide inhalation of short duration.
In the form of Pethidine 50 to 100 mg intramuscularly 2 to 1_ hours before the delivery

10 cm below the maternal introitus. The duration of labor maternal anesthesia, analgesia and birth weight of the two groups of infants were comparable. The data is listed in Table I

Cardiac catheterization by the transumbilical approach was conducted in the laboratory where room temperature was maintained between 24 to 26 C No sedation or anesthesia was used, and all infants breathed room air during the entire procedure. Rectal temperature was obtained just before and after the termination of each study The maximum difference between the two body temperature readings was less than ° (range of 34.5 to 36.5 C). A no 3 or 8 polyethylene feeding tube (Sterilon Corp.) containing 2 side holes 6 to 7 mm apart was inserted into the umbilical artery and advanced in retrograde fashion into the aorta up to a distance of 4 to 8 cm from the umbilical ring where at this level, its tip was generally located in the aorta upstream of the ductus arteriosus (proximal aorta), or in the pulmonary artery entered via the ductus arteriosus. The venous catheter was likewise advanced through the ductus venosus into the right atrium and, through the foramen ovale, into the left atrium. The catheter position was judged by the distance of its tip from the umbilical ring, the characteristics of the recorded pressure curves and the blood oxygen content. A detailed description of the catheterization technique has been reported elsewhere [1 2].

As the left atrium or proximal aorta was entered, blood samples were obtained anaerobically into a 2 ml heparinized and siliconized syringe provided with a mixing disc. Whenever both sites were entered, blood sampling was performed simultaneously Each blood sample was analyzed within 10 minutes after withdrawal for pH, pCO standard bicarbonate buffer base and base excess using the micro Astrup equipment [15]. The latter 4 values were derived from the Siggaard Andersen curve nomogram [16]. In addition, blood oxygen tension was determined by the polarographic method [5] using a Clark type electrode (E 5044, Radiometer Copenhagen) and blood oxygen saturation by reflection photometry using a Kipp-Zonen hemoreflector unit (1959 model). In 7 cases belonging to the early clamped group, oxygen saturation was derived from the observed pO values using an oxygen

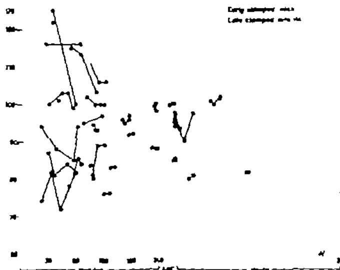


Fig. 1 Scattergram of left atrial or proximal aortic blood oxygen tensions of 118 normal term newborn infants with early and late cord clamping, during the first 24 hours of life. The pO values of the early clamped infants are significantly higher than those of the late clamped infants during the first three hours. Points connected by solid line indicate serial measurements from one infant.

dissociation curve [10] to avoid withdrawal of excessive blood samples. The pH pCO and pO_2 values were corrected to body temperatures using the correction factors of Rosenthal [14] and Dill [6]. Hematocrit values of each blood sample were measured by microcapillary technique.

Blood btained from the descending aorta as not included in this study to void samples with possible venous admixture from a potential right-to-left shunt t the ductal (arteriosus) levels.

When both left trial and proximal aortic blood samples were ailable, the erage of the two values were used. The maximal difference between the left atrial and proximal aortic blood samples for the different parameters were as follows: pO 7 mm Hg oxygen saturation 3%, pCO −2 mEq/1

N complications were encountered during and after the studies. N prophylactic antibiotics were used and ll infant ere in good condition t the time of ischarge

Results

As shown in Fig 1 infants with early cord lamping (E.C.) had significantly higher left atrial or proximal aortic blood oxygen tension (pO_2) than the late clamped (L.C) infants during th first three hours of lif At age 25 t 00 minutes, the E C infants had an average arterial pO_2 of 100 m Hg and at age 61 to 180 mi utes 98 mm Hg In contrast the L.C infa t had blood pO of 81 7 and 84 0 mm Hg du ring these age periods respectively Th ese differences are highly significant (p 0 001) From three to 24 hours of age the pO_2 values of the early and lat lan ped infants were comparable Stan dar l student t test was employed f r all statistical analysis.

In spite of the differences in pO_2, the

TABLE 2. *Arterial blood gas tension and acid base status o*

pO – Partial xygen te
Values are expressed in means ±

	pO² (mm Hg)				O² saturation (%)				pCO² (mm Hg)		
	25–60	61–180	181–300	301 to 24 hr	25–60	61 180	181–300	301 to 24 hr	25–60	61 180	181–300
Late clamped group	81.7 ±2.67 (9)	84.0 ±1.9 (26)	92 ±2.0 (21)	89 ±0.37 (39)	96.0 ±0.37 (8)	96.5 ±0.50 (21)	96.3 ±0.50 (19)	96.7 ±0.26 (41)	30.2 ±0.96 (8)	31.3 ±0.67 (13)	30.3 ±0.50 (31)
Early clamped group	100 ±3.6 (12)	98 ±1.9 (23)	94 ±2.1 (7)	91 ±2.4 (9)	96.9 ±0.57 (9)	96.3 ±0.39 (20)	97.5 ±0.65 (4)	97.3 ±0.60 (8)	27.1 ±0.89 (12)	27.– ±0.88 (19)	30.3 ±2.13 (7)
P	< .001	< .001	> 0.5	> 0.5	> 0.5	> 0.5	> 0.5	> 0.5	< .025 > .010	< .001	< 0.5

blood oxygen saturation of the E.C and L.C infants were the same This is not unexpected since the observed pO_2 values were situated in the flat segment of the oxygen dissociation curve.

A significant difference in blood carbon dioxide tension (pCO_2) between the E.C and L.C. infants was also observed during the first three hours of age (Fig 2) The L.C infants had a higher pCO_2 (30.– mm

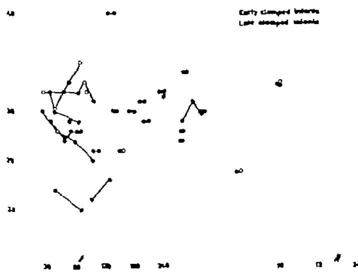


Fig. 2. Scattergram of left atrial or proximal aortic blood carbon dioxide tensions of 113 normal term newborn infants with early and late cord clamping, during the first 24 hours of life. The pCO values of the late clamped infants are significantly higher than those of the early clamped infants during the first three hours. Solid lines connect serial measurements from one infant

ewborn infants during the first 24 hours of age (age in minutes)

CO_2 Carbon dioxide tension.
Numbers of observations are given in parentheses.

	pH				Standard bicarbonate mEq/l				Base Excess mEq/l			
	5–60	61–180	181 300	301 t 24 hr	25–60	61 180	181 300	300 to 24 hr	25–60	61 180	181–300	301 to 24 hr
	7.373 ±0.0 (9)	7.250 ±0.007 (25)	7.345 ±0.009 (21)	7.370 ±.005 (40)	19.7 ±0.31 (8)	18.8 ±0.34 (23)	18.0 ±0.33 (22)	18.4 ±0.025 (40)	−6.3 ±0.49 (8)	−7.4 ±0.33 (23)	−8.1 ±0.46 (22)	− .6 ±0.33 (40)
	7.342 ±0.07 (11)	.260 ±0.004 (21)	7.365 ±0.019 (7)	7.396 ±0.016 (9)	17.4 ±0.43 (12)	17.3 ±0.39 (20)	18.3 ±0.50 (7)	19.0 ±0.67 (9)	−9.3 ±1.03 (12)	−9.3 ±0.33 (20)	7.8 ±0.77 (7)	6.9 ±0.86 (9)
	0.5	>0.5	>0.5	>0.5	<.001	<.025 >.010	>0.5	>0.5	<.025 >.010	<.025 >.010	>0.5	>0.5

Hg at age 25 to 60 minutes, and 31.3 mm Hg at age 61 to 180 minutes) than the E.C. infants (27.1 mm Hg at age 25 to 60 minutes and 27.. mm Hg at age 61 to 180 minutes).

Blood pH of E.C. and L.C. infants did not differ significantly. The values during the first 5 hours of life revealed slight acidosis; the average pH ranged from 7.250 to 7.370. At 24 hours of age, this had risen to normal levels.

During the first 3 hours of life the early clamped infants had significantly lower standard bicarbonate and higher buffer base deficit (larger negative base excess values) than the late clamped infants (Table . and Fig. 3). From 3 to 24 hours of age the standard bicarbonate and base excess values of the two groups of infants were no longer significantly different.

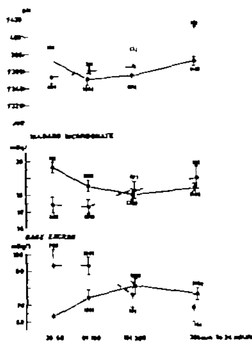


Fig. 3. Left atrial or proximal aortic blood pH, standard bicarbonate, and base excess of 37 early clamped and 31 late clamped infants during the first 24 hours of life. Open circle connected by solid lines are the mean values (± SE) of the late clamped infants while the closed circles connected by dotted lines are those of the early clamped infants. The statistical analyses of the values are presented in Table 3. Number of observations are enclosed in parentheses.

TABLE _ *Arterial blood gas tension and acid base status*

pO = Partial oxygen t
Values are expressed in means ±

pO² (mm Hg)				O² saturation (%)				pCO² (mm Hg)		
23–60	61–180	181 300	301 t 24 hr	23–60	61–180	181 300	301 to 24 hr	25–60	61–160	181–300
Lat clamped group										
81 7 ±2.67 (9)	84.0 ±1.9 (20)	93 ±2.0 (21)	89 ±0.27 (39)	96.0 ±0.37 (5)	96.5 ±0.50 (21)	96.3 ±0.50 (19)	96.7 ±0.26 (41)	30.2 ±0.96 (8)	31.3 ±0.67 (13)	30.3 ±0.80 (21)
Early clamped group										
100 ±2.6 (12)	98 ±1 9 (23)	94 ±_1 (7)	91 ±2.4 (9)	96.9 ±0.57 (9)	96.2 ±0.30 (20)	97 5 ±0.65 (4)	97.3 ±0.60 (6)	27 1 ±0.80 (12)	27.2 ±0.88 (19)	30.3 ±2.15 (7)
P										
< .001	< 001	> 0.5	> 0.5	> 0.5	> 0.5	> 0.5	> 0.5	< 023 > .010	< .001	< 0.5

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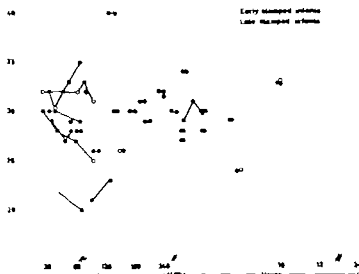


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early versus late cord clamping at birth. During the first three hours of life the late clamped infants had a lower paO_2 and higher $paCO_2$ than the early clamped infants.

During the first 5 hours of life both early and late clamped infants had mild metabolic acidosis. The blood pH was the same in both groups in spite of the lower pCO_2 level in the early clamped infants. There was in addition lower standard bicarbonate content and greater buffer base deficit in the early clamped infants.

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References

1. ARCILLA, R. A. OH W LIND J and GESSNER, I. H.: Pulmonary arterial pressures of newborn infants born with early and late clamping of the cord. *Acta Paediat Scand* *55* 305, 1966.
2. ARCILLA, R. A., OH, W LIND J and BLANKENSHIP W Portal and atrial pressures in the newborn period. A comparative study in infants born with early and late clamping of the cord *Acta Paediat Scand, 55* 615, 1966
3. ARCILLA, R. A., OH, W WALLGREN, G HANSON J GESSNER, I H. and LIND J D_2 indicator studies in the newborn period. II Hemodynamic features of infants born with early and late cord clamping. *To be published*
4. BUCKELS, L. J and USHER, R.: Cardiopulmonary effects of placental transfusion. *J Pediat, 67* 239 1965.
5. CLARK, L. C. HELMSWORTH, J A KAPLAN S, SHERMAN R. T and TAYLOR, Z.: Polarographic measurement of oxygen tension in whole blood and tissue. *Surgical forum of the American College of Surgeons* W B Saunders, Philadelphia, 1953
6. DILL, D B *Handbook of Respiration* W B Saunders, Philadelphia, 1958.
7. GANDY G GRANN L., CUNNINGHAM, N ADAMSONS, K JR., and JAMES, L. S The validity of pH and pCO measurements in capillary samples in sick and healthy newborn infants *Pediatrics, 34* 192, 1964.
8. OH, W LIND J and GESSNER, I. H The circulatory and respiratory adaptation to early and late cord clamping in newborn infants. *Acta Paediat Scand, 55* 17 1966.
9. OH, W WALLGREN G LIND J and HANSON J Lung compliance and functional residual capacity in the newborn infants in relation to placental transfusion *To be published.*
10. OH, W ARCILLA, R. A. and LIND, J Arterial blood oxygen desaturation curve of newborn infants. *Biol Neonat 9* 241 1966.
11. OH, W OH, M A. and LIND J Renal function and blood volume in newborn infant related to placental transfusion. *Acta Paediat Scand, 55* 197 1966.
12. OLIVER, T K., JR., DEMIS, J A. and BATES, G D Serial blood gas tensions and acid base balance during the first hour of life in human infants. *Acta Paediat Scand 50* 346, 1961
13. REARDON H., BAUMAN M. L. and HADDAD E. J Chemical stimuli of respiration in the early neonatal period. *J Pediat 57* 151 1960.
14. ROSENTHAL, T B.: The effect of temperature on the pH of blood and plasma in vitro. *J Biol Chem, 173* 25, 1948.
15. SIGGAARD ANDERSEN, O ENGEL, K., JORGENSEN K. and ASTRUP P A micromethod for determination of pH, carbon dioxide tension, base excess and standard bicarbonate in capillary blood. *Scand J Clin Lab Invest 12* 172, 1960
16. SIGGAARD ANDERSEN O The pH-log pCO_2 blood acid base nomogram revised. *Scand J Clin Lab Invest, 14* 598, 1962.
17. USHER, R., SHEPHARD, M. and LIND, J The blood volume of the newborn infant and placental transfusion. *Acta Paediat Scand, 52* 497 1963.
18. WEISBROT I. M., JAMES, L. S PRINCE, C E. HOLADAY D A. and APGAR, V Acid base homeostasis of the newborn infant during the first 24 hours of life *J Pediat, 52* 395, 1958.

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A study of Vitamin D Metabolism in Idiopathic Hypercalcemia of Infancy

by S G MANIOS and ILSE ANTENER

Idiopathic hypercalcemia of infancy first described in 1952 [10 27] is a rather rare condition. It is characterized mainly by gastrointestinal troubles, renal dysfunction, growth retardation and hypercalcemia and in its severe form, by marked osteosclerosis and mental retardation.

While the pathogenesis of the disease is relatively clearly defined its basic etiology is still not well established. The hypercalcemia, which explains the majority of the symptoms of the disease (renal damage hypertension, osteosclerosis etc.) is due to increased calcium retention, as has been shown by calcium balance studies [14 28 38] It is presently thought that the primary cause of hypercalcemia is a defect in the metabolism of vitamin D or a hypersensitivity to this vitamin [4 5 7 11 13 25 29 36 37]. Definite proof of such a defect is lacking however and its nature remains obscure

Vitamin D metabolism in this disease has not been thoroughly investigated to date This is not only due to the rarity of the disease especially in its severe form when one would expect the disturbance to be most apparent but also to the difficulties involved in the quantitative determination of vitamin D Such an investigation is further complicated by the limited knowledge of the different phases of normal vitamin D metabolism (mode of action at the cellular level, mechanism of its inactivation and elimination from the body) The scarcity of determinations of serum vitamin D activity in infants with hypercalcemia and the discrepancies in the reported results to date are not conclusive as to the exact role vitamin D plays in this disease

The purpose of this paper is to report the results of determinations of serum vitamin D activity both before and after the administration of a physiological dose of vitamin D in two patients with the severe form of idiopathic hypercalcemia. The data obtained provide evidence for the existence of an inborn error of vitamin D metabolism with defective inactivation of the vitamin D in idiopathic hypercalcemia

Subjects and Methods

The subjects of this investigation, two uniovular female siblings (T P and V P), were admitted to the Pediatric Clinic of the University of Thessaloniki for the second

TABLE 1 *Clinical and radiological features in the cases of idiopathic hypercalcemia at 23 months of age.*

Clinical and radiological findings	Case 1	Case 2
Weight in g	4600	4380
Height in cm	64	66
(3rd percentile at 24 months: 80.1)		
Head circumference in cm	42	42
Growth retardation	+	+
Malnutrition	+	+
Mental retardation	+	+
Psychomotor age with the Brunet Lezine test in months	6–7	6–7
Renal insufficiency (polyuria and azotemia)	+	+
Blood pressure	120/85	130/90
Microcephaly	−	+
Convergent strabism		+
Under-developed mandible	−	+
Osteosclerosis	+	+

time on April 20, 1964, because of poor physical and psychomotor progress. They were the products of an uneventful triple pregnancy born in M y 1962, at the end of the seventh gestational month, with birth weight 1700 g and 1600 g respecti ely

The third f these triplets, also female as affected by a mild and asymptomati form of idiopathic hypercalcemia in an inac tive phase t the time of diagnosis. U for tunately w have been unable to study this child.

A complet description of these cases of idiopathic hypercalcemia, up to the ge of 26 months, has been reported previously [30].

The clinical and laboratory data, at the time of diagnosis, were characteristic f the severe form of idiopathic hypercalcemia. The data, similar in both patients, is sum marized in Tables 1 and 2.

Both children presented a marked physi cal and mental retardation, malnutrition renal insufficiency with azotemia, polydip-sia, polyuria, hypertension, microcephaly con ergent strabism, underdeveloped mandi ble osteosclero- and elevation of the serum calcium.

The osteosclerosis was less pronounced in both patients at the time of the present investigation (Jul 1964) than in the past as shown by X ra taken at the ages of 13 months and 19 months. Two months later there was a *further improvement in this* condition and t the ge *of* 29 months the density of the bones wa almost normal in both patients.

The concentration of serum calcium un til June 1964 had aried in Case 1 from 12.6 mg to 15.2 mg, and in Case 2 from 16.6 mg to 18.2 mg/100 ml. It started falling progress ely from July onwards and on July 26 was 13.5 mg and 14.8 mg/100 ml, on September 4, 12.5 mg and 13.2 mg; on September 22, 12.8 mg and 13.0 mg; and on October 12, 11 6 mg and 12.1 mg/100 ml respectively

Despite the radiological and biochemical improvement, the other symptoms remained unchanged. The anorexia for solid foods persisted and the mental retardation, hyper tension, renal insufficiency and growth failure showed no ppreciable change.

On April 23 all supplemental itamin D was discontinued and feeding with unforti fied whole cow milk was instituted. During this investigation, and until the patients discharge from the Clinic on October 20 1964, both patients received 600 ml to 800ml of milk daily A treatment of 5 mg Predni-solon daily in two divided intra-muscular doses, was begun on September 3 and was discontinued on October 15.

Exogenous vitamin D intake. The vitamin D intake from birth until all sources of vitamin D were removed from the diet at the age *of 23 months, is summarized in Fig 1* From birth onwards, both patients were nourished almost exclusively with milk, owing to their marked anorexia. They were given 300 to 1000 ml of arious dry milk formulas daily the milk being fortified w th not less than 400 IU vitamin D per lit r diluted milk. Both patient received orally at the end of the first and third month, one massive dose of 300,000 IU of

vitamin D and another of 600 000 IU at the end of the fifth month. The patient V P received an additional dose of 300 000 IU at 20 months of age. In addition they w re given a polyvitamin preparation providing about 800 IU of water-soluble vitamin D per day as follows: patient T P continuously during the periods from to 6 and from 19 to 23 months of age and intermittently from 6 to 19 months; the patient V P continuously during the periods from 2 to 6 and from 8½ to 13 months and intermittently from 6 to 8½ and from 13 to 23 months.

Study with vitam n D 700 IU of vitamin D in oil solution were given to each of the patients fo 4 days at the age of 27 months. Each dose was given mixed with 100 ml of milk through a plastic feeding tube. The serum vitamin D acti ty was determined on the day prior to vitamin D administra tion and 48 hours after the last test dose

Determination f serum vitamin D Sixteen Wistar ats, four rats each from four females, weighing approximately 32 g each wer placed in separate cages and fed the McCollum rachitogenic diet No 3143 (Calcium 1.2% Phosphorus 0.24%) for a period of three weeks. At the end of the third week they were divided into four groups of four rat each, using a Latin Square Each rat then received Ca^{45} Cl (0 13 μC/day) every y fo one week. During this radioacti e ^{45} treatment two of the four groups recei ed the vitamin D standard in arious concentrations, 0.2 IU and 0 4 IU for example and the third group received a serum extract in a vitamin D-free vegetable oil. The fourth group recei ed only the calcium. Substances were administered to the animals by means of a short tube attached to a tuberculin syringe After this week long healing period the rats were killed by anaesthetic, and their tibias prepared. The tibias were first dried and then incinerat d at 800°C in Quart crucibles. The ashes were dissolved in hydrochloric acid and the cal cium precipitated as calcium oxalate This residue was then centrifuged washed and dried. The calcium oxalate was ground in an agat mortar weighed in an aluminium cup

TABLE 2 *Laboratory findings in the 2 cases of idiopathic hypercalcemia at 23 months of age*

Laboratory data	Case 1	Case 2
Blood urea, mg/100 ml	90	75
Serum calcium, mg/100 ml	15.2	16.2
Serum sodium, mEq/l	130	134
Serum potassium, mEq/l	4.3	4.6
Serum chloride, mEq/l	104	106
HCO_3, mEq/l	17 7	18.00
Blood sugar mg/100 ml	110	105
Cholesterol, mg/100 ml	233	185
Inorganic phosphorus, mg/100 ml	5.8	5.3
Alcaline phosphatase (King Armstrong units)	13	13
Total serum proteins, g/100 ml	7 0	6 6
Serum albumin, g/100 ml	3.2	3.1
Specific gravity of urine	1003	1005
pH of urine	5–6	5–6
Urinary calcium, mg/24 hours	25	5.
Urinary potassium, mg/24 hours	450	10
Urinary sodium, mg/24 hours	375	225

suspended in chloroform and dried under an infra red lamp The activity was determined with TCG β-sensitive Geiger tube attached to a counter It has been shown [2 3] that the counts/min may be expressed as a linear function of the log dose of vitamin D between 0.2 and 0 6 IU and as a linear function of the lose itself between 0 and 0 2 IU The counts/min of the serum are then read off curv and IU itamin D_3/100 ml serum cal culated

Results

The serum vitamin D activity in our two patients and in 21 normal infants, aged 3 to 24 months, is presented in Table 3 and Fig 2 The mean value of the normal infants was 133 IU/100 ml of serum. The highest normal value was 355 IU (range 50 to 355 IU); however in the

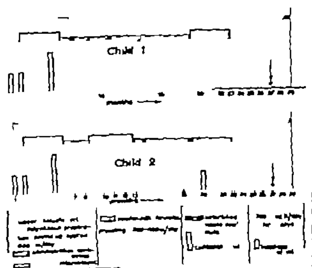


Fig. 1 Vitamin D intake of the hypercalcemic patients from birth until 23 months of age

majority of normal infants the serum vitamin D activity varied from 50 to 200 IU These values are comparable with those obtained in normal children by Warkany & Mabon [41] and Fellers & Schwartz [13] using the standard rachitic-rat bioassay procedure

The vitamin D level on the day prior to vitamin administration was elevated to times the normal mean value in the two hypercalcemic infants (Table 3)

The serum of the hypercalcemic infants contained 580 and 510 IU of vitamin D activity per 100 ml respectively A marked

TABLE 3. *Serum vitamin D activity before and after loading with 500 IU vitamin D (4 00 IU day) cases of idiopathic hypercalcemia*

	Serum vitamin D activity in IU 100 ml serum		
	Initial values	48 hours after the last test dose	Increase IU/100 ml serum
Case 1 (T P)	580 (95 confidence interval: 420 and 830 sup)	1 45*	065
Case 2 (V P)	520 (95 confidence interval: 370 and 60 sup)	1012*	493

A statistical examination has proved that these values are certainly higher than those given, as serum vitamin D concentration exceeded the standard curve.

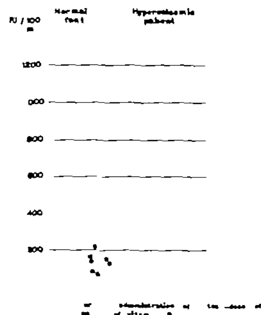


Fig 2. Serum vitamin D activity in 21 normal infants and in 2 hypercalcaemic patients. Mean value in 21 normal infants 132 IU (range 50–255 IU)

elevation of the serum vitamin D activity was found 48 hours after the last test dose of vitamin D

The levels increased from 590 to 1 45 IU in the first patient and from 520 to 013 IU in the second corresponding to a in the initial levels of 665 and 493 units respectively

Discussion

The etiologic role of vitamin D in idiopathic hypercalcemia is suggested by the similarity of the principal clinical and biological features in vitamin D intoxication and in this syndrome.

This hypothesis is also supported by epidemiological [16] and clinical [4 14, 18 28 38] observations concerning the effects of vitamin D intake on the disease incidence and its evolution.

A true vitamin D intoxication as the major cause of the disease cannot be accepted, however even in those few cases in which an excessive vitamin D intake is a proven fact

The vitamin D intake in the majority of the cases reported is not generally considered to be excessive and is often less than the recommended amount. The daily requirement for normal growth and prevention of rickets in infancy is estimated to be 400 to 800 IU [9 23]; it may vary however from 400 to 500 IU [33]

The vitamin D given to our patients, especially during the first 5 months of life is certainly in excess of the ordinary dosage. These doses, however cannot be considered toxic, especially for premature infants requiring large prophylactic doses of vitamin D according to some authors [8 32]

Zelson [42], who has given 600 000 IU of vitamin D in single doses to premature babies, has not observed that it had an injurious effect

To explain the appearance of the disease in infants receiving moderate quantities of vitamin D Bongiovanni *et al* [4] have stated that the syndrome may represent an expression of hypersensitivity to vitamin D

Fyfe [15] demonstrated a high concentration of vitamin A in the serum with a greater than normal rise after oral administration of vitamin A, of patients with idiopathic hypercalcaemia. He suggested that there might be a concomitant defect in the metabolism of vitamin D

Forfar *et al* [14] have proposed an abnormality in the metabolism of cholesterol with production of abnormal toxic sterols with vitamin D activity

All the above opinions certainly con

stitute a simple hypothesis. For this reason alone the importance of a direct investigation of the vitamin D metabolism in affected infants becomes obvious.

To our knowledge excessive serum vitamin D activity (500–6000 IU /100 ml) was found previously by Fellers & Schwartz [13] in 3 cases of the severe form of the disease, by Smith *et al* [36] in one similar case, and by Lang & Elard in another [26]. A small increase (360 IU/100 ml) was also found in one intermediate form of the disease by Stephan & Hövels [39]. Since the amounts of vitamin D received by these patients were moderate or below the normal requirements, these levels cannot be attributed to the exogenous vitamin D administration. The initial concentrations found in our patients was also abnormal and was not directly related to exogenous vitamin D intake. We have obtained levels of the same order of magnitude [17] in normal infants, with the administration of 240,000 IU of vitamin, within a period of two weeks.

Contrary to this our patients, during the 3 months period preceding this investigation, had not received any amount of vitamin D; similarly with the exception of the dose of 300,000 IU which the second patient received 6 months before the serum vitamin D assays, the mean daily intake in both patients, from the age of 5 to 23 months, was less than 1200 IU.

On the other hand, we have shown in a previous study [17, 21] that in normal as well as in rachitic children after a single oral dose of 600 000 IU the serum vitamin D activity which increased 24 hours following the administration from 1800 to 3600 IU fell below 750 IU within less than 20 days.

In contrast to the above-mentioned findings, other investigators [6, 19, 24, 25, 31, 35] reported normal serum vitamin D activity (100–300 IU/100 ml) in more than 15 patients with the severe or the mild form of the disease. Normal levels were found also by Thomas *et al* [40].

It is of interest to note that the vitamin D assays in many of these cases [6, 24] were performed during the inactive phase of the the disease, when the concentration of calcium in the serum had already returned to normal.

Regardless of this we believe that a defect in the metabolism of vitamin D cannot be excluded without a dynamic and complete exploration of its metabolism simply because a normal vitamin D level was found in the serum. A disturbance of vitamin D action at the cellular level cannot also be excluded. This becomes obvious from the results obtained in the case of Kenny *et al* [25] as well as in those of our patients. The administration of a physiological dose of vitamin D in the patient of Kenny *et al.* produced a marked elevation, from 300 to 800 IU of serum vitamin D.

A similar abnormal response was found in both of our cases, in whom an analogous rise was obtained after the administration of a smaller total dose during a shorter period of time.

A comparable elevation of the serum vitamin D activity has been obtained [17, 21] in normal as well as in rachitic infants with a dose 50 to 80 times greater than that stated above.

Thus, these findings give evidence to support the existence of a defect in the degradation or inactivation mechanism of vitamin D with subsequent accumula-

tion of endogenous or exogenous vitamin D.

Such a defect is compatible with the persistence in some cases [13, 36] of high serum vitamin D content for more than 12 months following a regimen without added vitamin, a period beyond which exogenous vitamin might persist normally. This also provides an explanation for the appearance of the disease in infants receiving moderate doses of vitamin D.

The monozygotic origin of our two sibling patients is well established [30]. This as well as the familial incidence of the disease in a few earlier reported cases [14, 22, 25] provides evidence favouring the possibility that the defect is genetically determined.

The physiological mechanism of vitamin D inactivation is still unknown. Experimental data suggests that vitamin D is inactivated in the liver [12].

Studies on adrenocortical function in our cases of idiopathic hypercalcemia reported elsewhere [30] and similar observations of others [1] suggest a primary adrenocortical deficiency or some abnormality in the metabolism of adrenal cortical steroids.

Animal experiments have shown that 30 min after the administration of vitamin D the majority of vitamin D is found in the adrenals, from where it disappears within 1 to 2 hours [34].

It is also of interest to note that adrenocortical hormones and vitamin D substances are all members of the steroid chemical group and both can be derived from cholesterol [20]. Nothing essential however is known about the possible interference of adrenocortical function with normal vitamin D metabolism in particular we have no knowledge at all if the adrenal cortex normally participates in the process of vitamin degradation.

Nevertheless, in the light of the aforementioned findings, the possibility of a pathogenic relationship between the abnormal metabolism of vitamin D and the adrenal cortex in idiopathic hypercalcemia cannot be excluded.

Summary

Serum vitamin D activity was determined in two cases of severe form of idiopathic hypercalcemia and the effect of a physiological dose of vitamin D was studied on this condition. The patients, 26 months of age, were uniovular female siblings, products of a triple normal pregnancy. Serum vitamin D activity was determined by a modification of the standard rachitic-rat bioassay method using radioactive calcium. The levels prior to the vitamin D test-dose, in comparison with those found by the same method in 21 normal infants, was markedly elevated, about 4 times the mean normal value.

A significant rise of the initial values was found 48 hours after the last test dose of vitamin D_2.

These findings suggest that the primary etiologic factor in idiopathic hypercalcemia, at least in some cases, is a genetically determined defect in the degradation mechanism of vitamin D.

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References

1 Anderson J, Brewis, E. G. and Taylor, W Adrenocortical function in hypercalcemia of infancy Arch Dis Childh, 32 114, 1957
2. Antener, L: Quantitative biologische Bestimmung von Vitamin D in der Ratte mit Hilfe von Ca[45] Abstracts of Papers. IV International Congress of Biochemistry Wien, 1958, Vol. VIII, p. 90.
3. Antener, L, Gentil, G and Pfeffer, J To be published in Arch Franç Pédiat
4. Bongiovanni, A. M., Eberlein W R. and Jones, I. T Idiopathic hypercalcemia of infancy with failure to thrive report of 3 cases, with a consideration of the possible etiology New Engl J Med, 257 951 1957
5. Bonham-Carter, R. E. D., Dent C. E., Fowler, D I. and Harper C. M: Calcium metabolism in idiopathic hypercalcemia with failure to thrive. Arch Dis Child, 30 399 1955.
6. Cuthbertson W F G The vitamin D activity of plasma of children with idiopathic hypercalcemia. Brit J Nutr 17 427 1963.
7 Daeschner, G L. and Daeschner, C W Severe idiopathic hypercalcemia of infancy Pediatrics, 19 362, 1957
8. Davidson L. T Merritt K and Chipman S: Further studies of viosterol in the prophylaxis of rickets in premature infants. Amer J Dis Child, 51 594, 1936.
9 Editorial: Hypercalcaemia in infancy Lancet I 1032, 1956.
10. Fanconi, G., Girardet P Schlesinger, B., Butler, N and Black, J Chronische Hypercalcämie, kombiniert mit Osteosklerose Hyperazotämie Minderwuchs und kongenitalen Missbildungen. Helv Paed Acta, 7 314, 1952
11. Fanconi, G and Spahr, A. Beiträge zur Frage der idiopathischen Hypercalcämie Helv Paed Acta 10 156 1955
12. Fellers, F X.: Idiopathic hypercalcemia of infancy and vitamin D metabolism. Helv Paediat Acta, 14 463 1959
13. Fellers, F X. and Schwartz, R Etiology of the severe form of idiopathic hypercalcemia of infancy New Engl J Med 259 1050 1958.
14. Forfar, J O Balf C L Maxwell, G M and Tompsett S. L Idiopathic hypercalcemia of infancy: clinical and metabolic studies with special reference to the etiological role of vitamin D Lancet I 981 1956.
15. Fuhr, M. Vitamin A levels in idiopathic hypercalcemia. Lancet I 810, 1956.
16 General articles and news Hypercalcemia in infants and vitamin D Brit Med J II 149, 1956.
17 Gentil, G and Antener, I. Dosage de l'activité (antirachitique D du serum chez l'enfant normal et au cours des désordres phosphocalciques de l'enfance. Abstracts of Papers X International Congress of Pediatrics Lisbon, 1962.
18. Gilbert R. D G and Neill, D M Idiopathic hypercalcemia in infants with failure to thrive Lancet II 110 1954.
19. Gyorgy P.: Discussion in Fellers and Schwartz Amer J Dis Child, 96 4 6, 1958.
20 Holleman A. F and Richter, F Lehrbuch der organischen Chemie Walter de Gruyter & Gruyter & Co Berlin, 1961 p 491.
21 Illig R., Antener, I and Prader, A Die Wirkung von Dihydrotachysterin (DHT) bei Mangelrachitis und die antirachitische Aktivität des Serums nach Verabreichung von DHT und Vitamin D Hel Paediat Acta, 16 490 1961
22. Illig, R. and Prader, A.: Kasuistische Beiträge zur idiopathischen Hypercalcämie und Vitamin D-Intoxication. Helv Paediat Acta, 14 618, 1959
23. Jeans, P C Council on Foods and Nutrition Vitamin D JAMA 143 177 1950
24. Jéquier, M., Jéquier-Doge, P Salle, B and Bouchayer S Hypercalcémie idiopathique à forme sévère du nourrisson. Contribution à l'étude de l'évolution et du pronostic. Pédiatrie, 19 169 1964
25 Kenny F M Aceto T Purisch M Harrison H. E. Harrison, H. C and Blizzard R. M Metabolic studies in a patient with idiopathic hypercalcemia of infancy J Pediat, 62 531 1963.
26. Lang, K. and Klaerr W: Beitrag zum Bilde der chronischen idiopathischen Hypercalcämie. Z Kinderheilk 9 490 1957
27 Lightwood R and Payne, W W Discussion of British Paediatric Association Proceedings of the twenty third general meeting. Arch Dis Childh, 27 307 1952
28 Lowe, K G Henderson J L., Park, W W and McGreal, D A. The idiopathic hypercalcaemic syndromes of infancy Lancet II 101 1954
29 MacDonald W B and Stapleton T Idiopathic hypercalcemia of infancy Studies of the mineral balance. Acta Paediat Scand 44 339 1955
30 Mariot, S., Panagou M., Thaler, D and A Kotrban: Hypercalcémie idiopathique chez des triplés Exploration de la fonction hypophysocorticosurrénale à la corticotropine et la métopirone du raison étiopathogénique. Arch Franç Pédiat, 23 63, 1966.

31. MORGAN, H. G.: Personal communication, cited by Smith *et al.* [36].
32. PARK, E. A.: The use of vitamin D preparations in the prevention and treatment of disease. *JAMA, 111* 1179, 1938.
33. PAUTE, J. Données actuelles sur la vitamine D. *Actualités Pédiatriques, 2e Série.* Doin et Co. Publ., Paris, 1961.
34. RAOUL, Y. and GOUNELLE, J. C.: Localisation surrénalienne de la vitamine D après injection intraveineuse chez le rat. *CR Acad Sci, 161* 247 I 1958.
35. SCHMID, R., JUST, M. and STALDER, G. Ein Fall von idiopathischer Hypercalcaemie im Kindesalter verbunden mit einem renalen Kaliumverlust-Syndrom. *Arch. Kinderheilk, 163* 149, 1960.
36. SMITH, D. W., BLIZZARD, R. M. and HARRISON, H. E.: Idiopathic hypercalcemia. A case report with assays of vitamin D in the serum. *Pediatrics, 24* 258, 1959.
37. STAPLETON, T. and EVANS, J. W. J. Idiopathic hypercalcaemia in infancy. *Helv Paediat Acta 10* 149, 1955.
38. STAPLETON, T., MACDONALD, W. E. and LIGHTWOOD, R.: Management of idiopathic hypercalcemia in infancy. *Lancet I* 932, 1956.
39. STEPHAN, U. and HÖVELS, O. Beobachtung zur Klinik und Therapie der sogenannten idiopathischen Hypercalcaemie. *Mschr Kinderheilk, 111* 102, 1962.
40. THOMAS, W. C., MORGAN, H. G., CONNOR, T. B., HADDOCK, L., BILLS, C. E. and HOWARD, J. E.: Studies of antirachitic activity in sera from patients with disorders of calcium metabolism and preliminary observations on the mode of transport of vitamin D in human serum. *J Clin Invest, 38* 1078, 1959.
41. WARKANY, J. and MABON, H. E.: Estimation of vitamin D in blood serum. II. Level of vitamin D in human blood serums. *Amer J Dis Child, 60* 606 1940.
42. ZELSON, C.: Prevention of rickets in premature infants with parenteral administration of single massive doses of vitamin D. *J Pediat, 17* 73, 1940.

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Antenatal Diagnosis of the Anemia in Erythrobla.tosis

A Comparison between Spectrophotometry and Erythropoietin Determination in the Amniotic Fluid

by PER HAAVARDSHOLM FINNE

Since the introduction of premature delivery in the treatment of selected cases of erythroblastosis, the antenatal diagnosis has become increasingly important. The intrauterine fœtal transfusion in prevention of fœtal anemia and intrauterine death [3 4, 14], has also increased the need for an exact antenatal prediction of the degree of the hemolytic disease In 1956 Bevis [ ] reported spectrophotometric investigations on the amniotic fluid in erythroblastosis, and since then several papers confirming the value of spectrophotometric examination of the liquor amnii in the antenatal diagnosis of erythroblastosis have been published [1 4 5 8, 10 11 1., 13 14 15] Previous investigations have shown that erythropoietin is demonstrable in amniotic fluid, and in cases with severely anemic fœtuses increased levels of erythropoietin have been found [6 9]. It was assumed that measurement of the erythropoietin content in the amniotic fluid before term could be of value when considering the problem of premature deli ery or intrauterine transfusions of the fœtus in Rh immunized pregnant women.

The present paper reports investigations carried out in an attempt to evaluate the diagnostic value of the two different procedures: spectrophotometry and erythropoietin determinations in the amniotic fluid in Rh-incompatible pregnancies.

Material and M thods

Amniotic fluid was collected by transabdominal amniocentesis before term or by rupturing the membranes during delivery or during cesarean section. The fluid upon aspiration was shielded from light, centrifuged immediately for 30' t 20 000 *g* and the spectral absorption curve between 400 and 600 mμ was determined using a Zeiss spectrophotometer and 1 cm cuvettes. The results were plotted on semilogarithmic paper and the height of the 450 mμ peak was determined and plotted in a diagram according t the instructions of Liley [11 12, 13].

The erythropoietin content of the amniotic fluid was determined using transfusion induced polycythemic mice as recipients and the Fe^{59} incorporation into red cells as parameter One ml of amniotic fluid was injected subcutaneously on tw consecutive days. As controls served mice injected with amniotic fluid from normal pregnancies at term [6].

Capillary hemoglobin and bilirubin was determined in the newborn infant immedi at ly after delivery

Results

Fig 1 shows the results of the erythropoietin determinations in the liquor amnii. It is seen that an abrupt rise in erythropoietin content is found when capillary hemoglobin falls below 11–10 g Also in cases with a milder degree of anemia, slightly elevated erythropoietin levels may be found, compared with levels in amniotic fluid from normal pregnancies.

Fig 2 shows the results of repeated erythropoietin determinations in some pregnancies. Rising erythropoietin levels were found in cases where severely anemic infants were delivered, thus indicating intrauterine hypoxia. The figures attached to the letters indicate the capillary hemoglobin at delivery

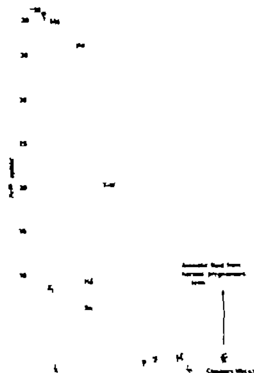


Fig. 1. The Fe^{59} uptake in polycythemic mice injected with amniotic fluid from Rh-immunized women and the corresponding hemoglobin values of the newborn infants. An abrupt rise is seen in erythropoietin level when capillary hemoglobin drops below 11–10 g/100 ml. In many cases of milder anemia slight elevation of the erythropoietin level is also seen compared to normal amniotic fluid at term. The letters attached correspond to those in Figs. 2, 3 and 5.

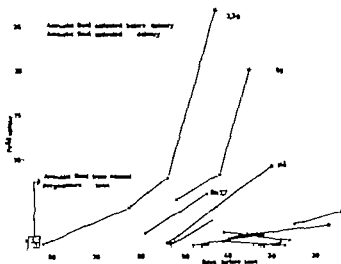


Fig. 2. The results of repeated erythropoietin determinations in the amniotic fluid. A marked increase in the erythropoietin level was found in some cases in which the fetus were severely anemic at delivery The figures attached to the letters indicate capillary hemoglobin at delivery



Fig. 3. Correlation between the optical density peak at 450 mμ and the capillary hemoglobin at delivery. The range of the peaks in some cases in which the fetus occurred to be Rh negative are also shown. The open circles with letters attached indicate cases in which markedly erythropoietin levels in amniotic fluid were demonstrable (Fig. 1). † indicates perinatal death.

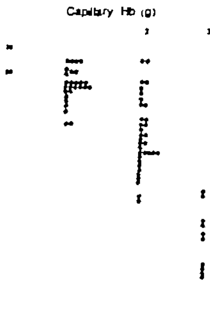


Fig. 4. Scattergram of the capillary hemoglobin values at delivery in the different zones. The greatest variation is found in zone ... One case in zone 3 showed normal hemoglobin at birth. This infant showed a greatly elevated serum bilirubin level at delivery 10.2 mg/100 ml.

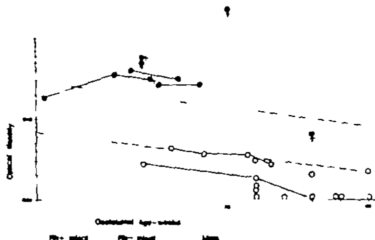


Fig. 5. The figure illustrates the spectrophotometric findings in some severely affected cases, in cases who died perinatally and in cases where the infants were Rh-negative. One case who died perinatally fell in zone 2, however death was not caused by erythroblastosis itself.

Fig 3 shows the correlation between the last spectrophotometric investigation of the liquor before delivery and the corresponding capillary hemoglobin of the infant at delivery The time between amniocentesis and delivery ranged from 1 to 10 days. The height of the peak at 450 mμ calculated as described by Liley [11] is plotted along the ordinate (logarithmic) and capillary hemoglobin along the abscissa.

Fig 4 shows a scattergram of capillary hemoglobin values based on the distribution in the different zones in which the 450 mμ peak fell, when plotted in the diagram of Liley [11]

Fig. 5 demonstrates the spectrophotometric findings in some severely affected cases, in cases who died perinatally and in cases where the infants were Rh negative (Liley s diagram)

Discussion

When considering the problem of premature delivery in the Rh immunized pregnant women information about the foetal hemoglobin level is of great interest Usually the clinical condition of the newborn shows a good correlation to the hemoglobin value in the infant The danger of prematurity has to be weighed against that of fœtal anemia, and the right time for delivery is when the danger of anemia exceeds that of prematurity The diagnostic value of the different procedures used in prediction of the fœtal anemia depends on the accuracy with which the fœtal hemoglobin level can be predicted.

The erythropoietin assays in the amniotic fluid show an abrupt rise in erythropoietin content when hemoglobin drops below 11–10 g At higher hemoglobin levels the erythropoietin content is more varying and the determinations consequently of less value in the antenatal diagnosis of the anemia of the newborn. However in many cases, even with milder degree of anemia, a slight increase in erythropoietin is found, indicating a raised fœtal red cell production compensating for the increased destruction by hemolysis. In cases with severe anemia, repeated assays may detect an increase in erythropoietin level as pregnancy progresses (Fig) The finding of an elevated or a rising erythropoietin level indicates that the fœtus is at risk. The prognosis in such cases is dubious. In some few cases, although severe anemia was present in the fœtus only slight or no increase in the amniotic erythropoietin level was found. It has previously been shown that meconium discharge may destroy the erythropoetic activity of the amniotic fluid [6]. This is the explanation of the cases reported here where the amniotic fluid was meconium stained.

The abrupt rise in erythropoietin content at 11–10 g may mainly be due to the fœtal anemia per se At this level anemia interferes with the oxygen supply to the fœtal tissue giving a hypoxia which in turn stimulates erythropoietin production to such an extent that a rise in amniotic fluid erythropoietin content is induced. In cord blood increased erythropoietin levels can be demonstrated at 13–11 g [7]. Circulatory and placental disturbances may also be contributory factors. In severe erythroblastosis the placenta becomes edematous and this may to some extent interfere with the gas exchange. Cardiac circulatory failure in the fœtus, due to the

anemia, may also contribute to the tissue hypoxia and thus give a rise in the erythropoietin production. The erythropoietin assays do not allow accurate prediction of the foetal hemoglobin level, it rather reflects the foetal tissue oxygenation. The cases demonstrated in Fig 1 can be separated into two groups. In the first one the hemoglobin levels were 11–10 g or lower and tissue oxygenation highly insufficient In these cases an increased erythropoetin content of the amniotic fluid was demonstrated. In the other group only small amounts of erythropoietin were detectable and the hemoglobin values were above 11–10 g. Other causes interfering with the oxygen supply to the foetus may give increased amniotic fluid erythropoietin levels such as preeclampsia, postmaturity and diabetes [7]

In Fig 3 is shown the optical density peak at 450 mμ in the last tracings before delivery and the capillary hemoglobin at delivery There is a reasonabl good correlation, as also found by ther investigators [11].

As demonstrated in Fig 4 there is some variation in the hemoglobin level in the different zones, especially in zone ? All cases with Rh negati e foetuses fell within zone 1 or on the border to zone (F g 5) The results of the spectrophotometric analysis seem to show that cases t risk give a marked rise in the height of the 450 mμ peak and are easy to detect even in a single tracing (Figs 3 and 5) These cases call for immediate intervention. Tracings falling in zone 1 indicate either an Rh-negative baby or a slight degree of hemolysis in the foetus. Tracings falling in zone are more difficult to interpret There is a great variation in the corresponding hemoglobin level of the foetus. Repeated amniocentesis is indicated for predicting the degree of the foetal anemia. The trend of the 450 mμ peak in repeated tracings gives some additional information about severity of the hemolytic disease [1]

As stated by Liley [13] normal amniotic fluid contains some amount of bilirubin and other pigments responsible for the peak at 450 mμ. There is however a fall in pigment concentration with increasing gestational age. The slope of the lines dividing the diagram into different zones indicates this decline. As demonstrated in Fig 5 repeated tracings of amniotic fluid from two pregnacies in which the foetuses were Rh-negative fell in with this

When comparing Figs. 1 and 3 it is interesting to note that cases which showed th highest peaks at 450 mμ also showed markedly increased erythropoietin levels in the amniotic fluid. This means that both findings suggest a hypoxic foetus and call for immediate intervention. Further it is seen that there is a better correlation between the result of the spectrophotometric examination and the capillary hemoglobin, than between hemoglobin and erythropoietin levels in the amniotic fluid.

In conclusion one may say that both erythropoietin estimations and spectrophotometry will detect severely anemic cases. For evaluation of moderately affected foetuses spectrophotometry is the more reliable procedure. Furthermore erythropoietin assays are time con uming and therefore not so well suited for routine use. If an easier and more rapid method could be found, assays of erythropoietin in the amniotic fl id could be a valuable

supplement to spectrophotometry For the moment spectrophotometry should be the method of choice. In selected cases amniocentesis and spectrophotometric analysis of amniotic fluid in erythroblastosis is found to be a safe and valuable antenatal procedure, helping to decide which patients require early delivery and at what time they should be delivered and which patients can go on to term and spontaneous delivery The complications of amniocentesis, although not common in experienced hands, should not be forgotten [14] The indications for amniocentesis should be based on other criteria such as case history father s zygosity for the Rh factor and mother s antibody titer

It should be added that all sorts of intra uterine hypoxia will give an elevation of fœtal erythropoietin production [7]. In cases with prolonged fœtal distress, as in preeclampsia, determination of amniotic fluid erythropoietin content could be of help in predicting the degree of the intrauterine fœtal hypoxia.

Summary

Spectrophotometry and erythropoietin determinations in the amniotic fluid have been used in the antenatal diagnosis of the anemia in erythroblastosis. An abrupt rise in the erythropoietin level was found when hemoglobin dropped below 11–10 g In cases with a milder degree of anemia only a slight or no elevation of erythropoietin levels, compared to normal amniotic fluid, were found. The height of the optical density peak at 450 mμ calculated as described by Liley [11] shows a reasonably good correlation to the capillary hemoglobin level at birth It is concluded that for routine use spectrophotometric analysis of amniotic fluid is the method of choice.

References

1 ALVEY J P Obstetrical management of Rh incompatibility based on liquor amnii studies. *Amer J Obstet Gynec*, *90* 769, 1964.
2. BEVIS, D C A.: Blood pigments in hemolytic disease of the newborn. *J Obstet Gynaec Brit Emp*, *63* 68, 1956.
3. BOWER, W A DROSE, V E. and BRUNS, P D Amniocentesis and intrauterine fetal transfusion in erythroblastosis. *Amer J Obstet Gynec 93* 822, 1965.
4. BOWMAN J M and FRIESEN R. F Multiple intraperitoneal transfusions of the fetus for erythroblastosis fetalis. *New Eng J Med 271* 03, 1964
5. BOWMAN J M. and POLLOCK, J M.: Amniotic fluid spectrophotometry and early delivery in the management of erythroblastosis fetalis. *Pediatrics*, *35* 815, 1965.
6. FINNE, P H.: Erythropoietin levels in the amniotic fluid, particularly in Rh-immunized pregnancies. *Acta Paediat Scand*, *53*, 269 1964.
7 FINNE, P H.: Erythropoietin levels in cord blood as an indicator of intrauterine hypoxia. *Acta Paediat Scand 55* 478, 1966.
8. FREDA, V J The Rh problem in obstetrics and new concept of its management using amniocentesis and spectrophotometric scanning of amniotic fluid. *Amer J Obstet Gynec*, *92*. 341 1965.
9 HALVORSEN S. and FINNE, P H. *Transfer of erythropoietin t amniotic fluid in Rh-immunized pregnant women. Brit Med J I* 1132, 1963.
10. LEWIS, F SCHULMAN H. and HA ABEL, T T Spectrophotometric analysis of amniotic fluid in erythroblastosis *fetalis. JAMA 190* 105, 1964.
11 LILEY A. W Liquor amnii analyses in the management of the pregnancy complicated by rhesus sensitization. *Amer J Obstet Gynec 82* 1359, 1961
12. — Errors in the assessment of hemolytic disease from amniotic fluid. *Amer J Obstet Gynec*, *86* 485 1963.
13. — The use of amniocentesis and fetal transfusion in erythroblastosis f talis. *Pediatrics*, *35* 836, 1965
14. QUEENAN J T and ADAMS, D W Amniocentesis A possible immunizing hazard. *Obstet Gynec 24* 530 1964.
15. WALKER, A. H. C and JENNISON R F Antenatal prediction of hemolytic disease of newborn. *Brit Med J II* 1152, 1962.

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Portal and Atrial Pressures in the Newborn Period

A comparative Study of Infants Born with Early and Late Clamping of the Cord[1]

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The circulatory adjustments in the newborn infant immediately following its birth are essential for a successful transition from uterine to extrauterine life. These changes generally consist of disappearance of the fetal intracardiac flow pattern, emergence of new vascular pathways and functional expansion of the cardiovascular bed. How these circulatory changes are influenced by the volume of the blood transferred from the placenta to the infant during the birth process is not precisely known.

This investigation is a part of a systematic study dealing with the physiologic adaptation of the newly born infant to placental transfusion. Since the time of clamping of the cord regulates the amount of placental blood transfer and, consequently the neonates blood volume [13, 19], comparison of the cardiovascular status of infants born with early and with delayed clamping of the cord has been undertaken. The present communication deals with a comparative study of the portal, right atrial and left atrial pressures during the first 14 hours of life in these 2 types of subjects.

Material and Method

Ninety three full term normal newborn infants, age ½ to 14 hours, were studied. The pregnancy in each case was uneventful, and the delivery was uncomplicated, through the vaginal route and in cephalic presentation. No medications were given to the mothers except in 10 cases who received 50 to 200 mg Pethidine intramuscularly and in 29 who had intermittent N O anesthesia.

In 34 cases, clamping of the cord was performed within 5 or, in few instances, 10 seconds after the delivery and in 59 other cases, it was done after cessation of the umbilical arterial pulsations [1, 0, 13, 19]. The former cases comprise the early-clamp (EC) group, and the latter the late-clamp (LC) group.

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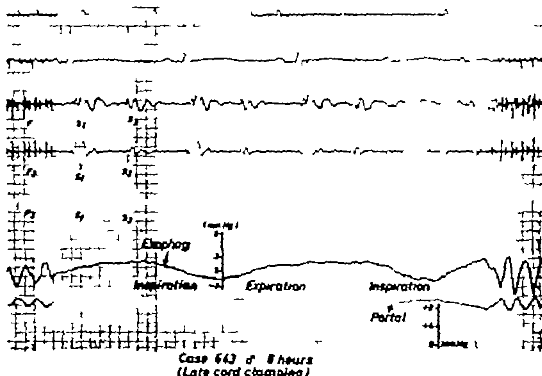


Fig. 1. Simultaneous intraesophageal and portal (sinus) pressures demonstrating their opposite respiratory pressure fluctuations. Electrocardiogram and phonocardiogram also recorded. Paper speeds 10 and 50 mm/sec.

All infants were considered in good condition at birth and at the time of our study. None required resuscitation or oxygen inhalation after birth. No abnormal cardiopulmonary findings were noted in any subject. There were 18 males and 16 females in the early-clamp group, and their mean birth weight was 3660 g (range: 2880 to 4270 g). There were 20 males and 10 females in the late-clamp group, and their mean birth weight was 3380 g (range: 2840 to 4680 g).

The hemodynamic investigation was done prior to the infant's first feeding, without anesthesia or analgesia, and with the infant breathing room air. A detailed account of the technic has been reported elsewhere (1). A 5 F or 8 F polyvinyl nasogastric feeding tube was inserted into the umbilical vein and advanced into the right atrium, left atrium and, occasionally, into either ventricle. A separate 5 F catheter was also introduced into an umbilical artery and advanced into the aorta, pulmonary artery or occasionally into either ventricle. Blood samples collected from various sites were analyzed for gas content, pH and hematocrit. The intracardiac position of the catheter was determined from the distance of its tip from the umbilical ring, from the pressure curves and from the blood oxygen content. Pressures were obtained with an Elema transducer and registered by a Mingograph 81 jet-writing recorder. Zero reference was set at the anterior axillary line. Mean pressures were obtained through electrical integration.

Pressure tracings were obtained during slow withdrawal of the venous catheter from

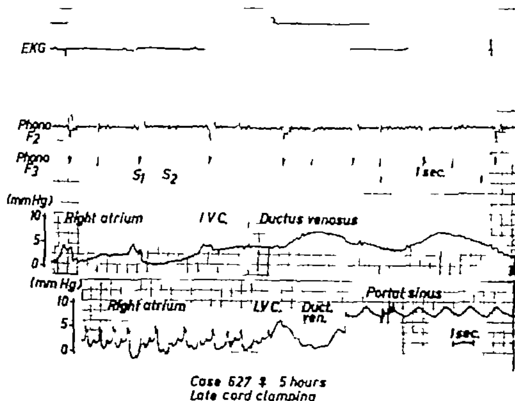


Fig. 2. Withdra al pressure tracings from right atrium to inferior vena cava and ductus enosus, to portal sinus area. Electrocardiogram and phonocardiogram also simultaneously recorded. Paper speeds 50 mm/sec. (pper tracing) and 10 mm/sec. (lower tracing). Note abrupt pressure difference between the ductus enosus and portal sinus.

the left atrium int the right atrium and then into the portal sinus area, interrupted only briefly by sampling at these sites. The catheter was then advanced back to the atrium whereupon the remaining procedures of the hemodynamic study not covered in the present report were resumed. Registration f the atrial-to-portal (and, vice versa) tracings was done only when the infant was quiet

The portal sinus tracing was identified from the right atrial to-portal withdrawal curve as that portion of the curv showing an abrupt rise of pressure devoid of venous pulsations and showing regular respiratory fluctuations (Figs. 1 and 2). The level during expiration was utilized to indicat the portal pressure. In many instances where withdrawal was done very slowly and t fast recorder paper speeds, a transitional segment inter posed between the typical right trial and portal curves could often be recognized. This was identified to be the ductus venosus (and inferior vena cav) tracing, haracterized by pressure of roughly the same level as that of the right trium and minimal venous pulsations (Fig. 2).

The height of the and v wa es, "x level and mean pressures were determined in the atrial pressure curves, taking the average of pproximately 10 consecut e cycles to correct for respiratory fluctuations. The previously reported data on 13 atrial

TABLE 1 *Portal and atrial press*

Subject		Hematocrit	Portal pressure (mm Hg)		
Age group	Cord clamping	mean ±1 S.E.M.	No. subjects	mean ±1 S.E.M.	Differe P val
< 1 hour	Early	48.3 ± 2.0	3	7 3 ± 0.60	$P > 0.$
	Late	54.3 ± 1.95	2	7 0 ± 1.0	
1–3 hours	Early	49 4 ± 0 90	16	6.3 ± 0.53	$P > 0.$
	Late	57 7 ± 1.88	11	6.5 ± 0.53	
3–6 hours	Early	48.3 ± 3.2	4	5.0 ± 0.76	$P > 0.$
	Late	58.7 ± 1.27	21	6.6 ± 0.44	
> 6 hours	Early	47.3 ± 4.6	4	4.4 ± 0.70	$P > 0.$
	Late	58.3 ± 1.11	5	6.1 ± 0.31	

pressure curves obtained during the first hour of life [2] have been included in the present series. A total of 91 atrial curves, each belonging to one subject, were analyzed.

Result

Of the 93 portal tracings studied, 24 had no accompanying atrial curves since the catheter could not be advanced into the right atrium. In the other 69 cases, a portal-right atrial pressure gradient averaging 5 6 mm Hg (range: 1.5 to 11 mm Hg) was consistently observed (Fig 3). The site of the pressure change was localized at the ductus venosus level (Fig)

The mean portal sinus pressure of all early-clamped (EC) infants was 6.3 mm Hg (±0 40 S.E.) and that of all late-clamped (LC) infants 6.5 mm Hg (±0.23 S.E.) There was no significant difference between the pressures of the types of subjects, considered as a whole or in individual age groups (Fig 3)

Aside from its relatively high pressure level, the portal sinus tracing revealed a regular undulating configuration consisting of a rise during inspiration and a fall during expiration. In several instances where simultaneous intraesophageal and intragastric pressures were obtained, the portal pressure fluctuations coincided with those of the intragastric curves but occurred in reciprocal fashion with those of the esophageal tracings (Fig 1) During quiet respiration, the inspiratory pressure rise ranged from 1 to 3 mm Hg in about 90 per cent of cases this was roughly 25 to 50 per cent that of the pressure fall in the corresponding atrial or esophageal tracings.

The right atrial and left atrial mean pressures have been plotted in a scattergram in Fig 4 In addition mean values at comparable age groups of the EC and LC infants have been superimposed. During the first 60 minutes of life, the atrial pressures of the LC infants were significantly higher than those of the EC infants ($P < 0.001$ see Table 1). After this period, the differences between the groups were no longer significant although the left atrial pressure remained somewhat higher in the LC infants. In contrast to the nearly flat atrial pressure levels of the EC

uring the first 14 hours of life

Right atrial mean pressure (mm Hg)			Left atrial mean pressure (mm Hg)		
No. subjects	mean ±1 S.E.M.	Difference P value	No. subjects	mean ±1 S.E.M.	Difference P value
8	0 ±0.3[illegible]	$P < 0.001$	6	4.[illegible] ± [illegible]59	$P < 0.001$
13	4.8±0.73		13	9.7±0 [illegible]1	
16	0.2±1.2	$P > 0.20$	10	4.2±0.4[illegible]	$P > 0.05$ < 0.10
11	1.0±0.66		10	5.7±0.63	
8	0 ±0.27	$P > 0.05$ < 0.10	3	2.8±1.43	$P > 0.20$
10	1.8±0.50		11	5.0±0.60	
8	−0.8±0.25	$P > 0.20$	3	3.0±1.14	$P > 0.10$
10	0.2±0.38		16	4.6±0.39	

subjects during the first 9 hours, those of the LC subjects showed a significant decline during the first hour (Fig. 4).

The pressure in the left atrium was always greater than that of the respective right atrium. This was true not only of the mean pressures but also of the amplitude of the various wave components. The pressure difference did not vary according to the actual atrial pressure level, and appeared to be uninfluenced by age. It was generally highest at the time of the "v" waves (Fig. 5). In addition, the configuration of the right atrial and of the left atrial pressure curves differed. The left atrial tracings revealed tall "v" waves with rapid ascending and descending limbs, as well as comparatively high "x" levels. This finding was especially prominent during the first hour. In contrast, the right atrial curves showed low "v" waves and "x" levels (Fig. 6). Accordingly, the rate of pressure rise of the left atrial "v" wave, as manifested by the slope of its ascending limb, was often [illegible] or more times that of the right atrial counterpart. These differentiating atrial pressure patterns were equally observed in both the EC and the LC subjects.

Discussion

At the time of its birth, the newborn infant is subjected to placental transfusion of varying magnitude depending upon the time when the umbilical cord is clamped [13, 19]. The relative size of this placental blood transfer is reflected by the difference in the blood volumes of newly born infants delivered with early and with late clamping of the cord. During the first 15 to 30 minutes of life, the blood volume (RISA method) of vaginally delivered late-clamped subjects has been observed to be approximately [illegible]5 to 30 per cent greater than that of infants whose cord has been clamped within few seconds after delivery [13, 19]. Since some degree of plasma shift to the extravascular tissues may have already occurred by this time, it is likely that this blood volume difference is even greater right after birth [19].

This rapid transfer of a large volume of blood necessitates an efficient homeostatic mechanism of the neonate. One may in

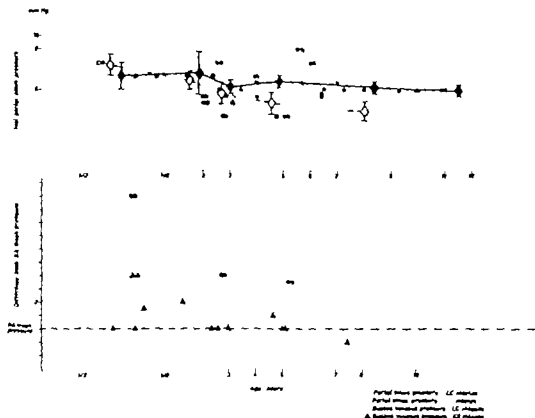


Fig 3. Portal (sinus) pressures of EC infants (open dot) and of LC infants (closed dot) plotted against age. Each dot belongs to an individual case. *Upper* graph shows the actual portal pressure. Mean values (±1 S.E.) are shown by the open diamonds (EC) and closed diamonds (LC) at the following age groups: <1, 1–2, –4, 3–6, 6–9 and >9 hours. The solid and broken lines join the respective mean values, and the vertical bars indicate 1 S.E. *Lower* graph shows the portal right atrial pressure gradient. Not minimal difference between ductus venosus pressure and right atrial mean pressure indicated by the zero horizontal dotted line.

criminate expansion of the highly distensible reservoir vessels of which the veins are the most important. It has been estimated that approximately 80 per cent of the total blood volume is confined in the low pressure region of the cardiovascular system comprising the veins, right heart chambers, pulmonary circuit and left atrium (including left ventricle in diastole) [18]. The veins and venules contain some 55 per cent [10] of the blood volume or even more [6, 22]. The regional distribution of blood in the newborn as well as the time it becomes stabilized following birth is not known. It is not unlikely that most of the blood resides in this low pressure region which is especially adapted to accommodate large increments in blood volume with minimal pressure change. This may perhaps explain the absence of a significant difference in the atrial pressures of the LC and EC infants after the first hour in spite of a substantial difference in their blood volumes. How

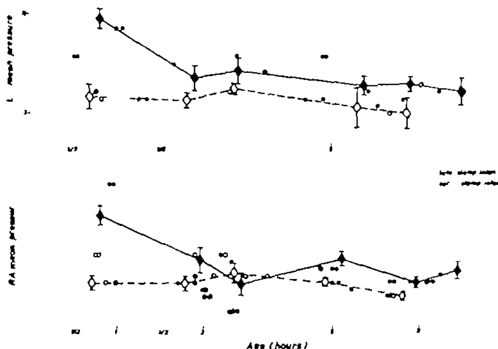


Fig. 4. Atrial mean pressure of EC infants (pen dot) and of LC infants (closed dot) plotted against age. Mean values (±1 S.E.) have been drawn using the same scheme and age grouping as that used in Fig. 3. Not high trial pressures of the LC subjects during the first hour and the much lower pressure levels thereafter

ever the high atrial pressures observed in the LC infants before the age of 1 hour suggest that there is still significant over loading of the circulatory system due to an as yet insufficiently expanded reservoir compartment relative to the suddenly augmented blood volume of the neonate and, perhaps to some extent madequate plasma shift. The subsequent atrial pressure drop observed in this particular group of subjects is in keeping with this hypothesis.

The portal pressures obtained in our series (LC subjects: m = 6.5 ± 0 23 S.E. mm Hg; EC subjects: m = 6.3 ± 0 40 S E. mm Hg) are comparable to those reported by thers [5 9] Mean values obtained from adult humans without liver disease by direct portal venous manometry during laparotomy or by occlusive hepatic vein catheterization, are also comparable rang mg from 5 to 8 mm Hg [10 14, 17 18 20] In all of the above mentioned studies, i.e. m the new born infants as well as in th adults, a pressure difference was always demonstrable between the portal vein and the vena cava or right atrium (Fig 3). This represents the perfusion pressure gradient which ppears to be an important determinant of liver blood flow since most

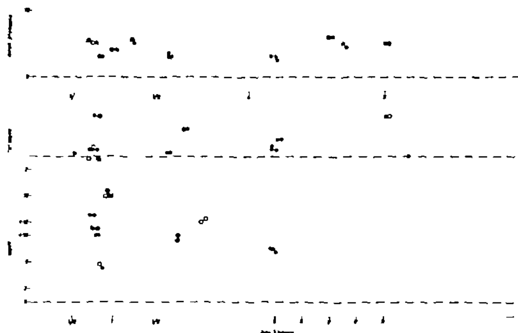


Fig. 5. Left atrial-right atrial pressure differences (in mm Hg) of the EC infants (open dot) and of the LC infants (closed dot) plotted against age. The atrial pressure differences have been categorized according to: mean pressure (upper graph), " wave (middle graph) and " wa (lower graph)

of the liver blood supply originates from the portal vein [3 7].

Umbilical venous pressures obtained at or immediately after birth are approximately 4 times those observed by us at age ½ hour or later. Pressures taken during cesarean section with the infants still in utero ranged from 17 to 34 mm Hg with mean of 24 to 26 mm Hg [8, 11]. Those obtained immediately after birth from vaginally delivered babies and recorded in between uterine contractions, showed similar pressure levels [12] Wallgren *et al* [1] also noted high umbilical venous pressures which dropped significantly after the onset of respiration.

It is not yet established when and to what extent post-natal expansion of the gastrointestinal vascular bed occurs as part of the overall circulatory adjustment of the newly born infant to its suddenly increased blood volume. Theoretically speaking, this should result in increasing portal blood flow and pressures. However our failure to observe a rise in portal pressure may be accounted for by the functional patency of the ductus venosus and, conceivably by some increase of the liver sinusoid vascular bed. The significance of a functioning ductus venosus in the human after birth is not well understood. The pressure gradient between the

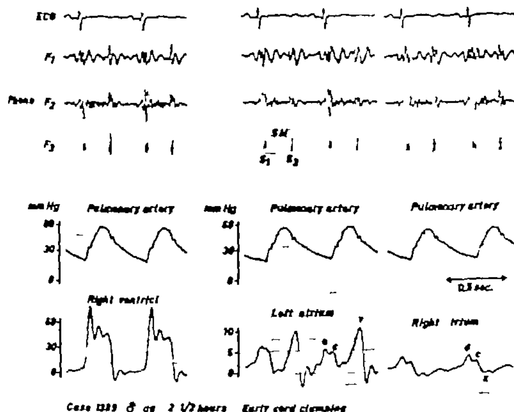


Fig. 6. Representative intracardiac pressure tracings registered simultaneously with phonocardiogram and electrocardiogram of newborn infant. Note difference in the contour of the right atrial and of the left atrial pressure curves.

portal sinus and the central veins appears to be localized at this level indicating significant narrowing of this structure. Nevertheless, indicator-dilution studies (2-catheter technic) on infants less than 24 hours of age indicate that a significant portion of the portal venous return still flows across this vessel directly into the inferior vena cava (unpublished data). The functional patency of this structure in the human neonate has also been demonstrated by angiocardiography [15]. Without this bypass channel, higher portal pressures may be expected at this age. This accessory portal venous outlet could easily account for the absence of a pressure difference between our EC and LC infants during the first hour. This difference was observed in the atrial tracings.

Burnard & James [4] have also observed higher atrial pressures in the newborn infants allowed maximal placental transfusion. The drop in atrial pressures shortly after birth has likewise been reported [2, 4]. The consistent pressure difference between the left atrium and right atrium is generally assumed to account for the functional closure of the foramen ovale

after birth. This LA-RA pressure differential did not appear to vary with age in our series. From our data, there appears to be no preferential time in the immediate newborn period (excluding the first ½ hour after birth which we have not studied) for significant right-to-left atrial shunting to occur.

The typical left atrial pressure curve of the newborn infant during the first hour consisting of tall "v" waves with rapid ascending and descending limbs was also observed in the older subjects although not as strikingly and with some individual variations. The hemodynamic significance of this finding has been discussed in a previous report (7). The prominent left atrial "v" wave has been attributed to the lower compliance of the left atrial chamber as compared to that of the right and, as a contributing factor, to the left-to-right shunt across the ductus arteriosus. The inability of the left atrium to enlarge and effectively accommodate the sudden increase in pulmonary blood flow following birth has also been pointed out. Since the "v" wave occurs at the time when the tricuspid and mitral orifices are closed, the influence of ventricular compliance and filling pressure upon its height and rate of pressure rise is presumably minimal. In none of our cases was an apical systolic murmur noted during auscultation or in the phonocardiogram. This speaks against the likelihood of mitral regurgitation in the genesis of the prominent "v" waves in these subjects.

The contour difference between the two atrial tracings is helpful in localizing the catheter site, especially in the absence of cardiac fluoroscopy during cardiac catheterization of the newly born infant. Unless there are other supporting clinical or laboratory data, prominence of the left atrial "v" wave should probably not be interpreted to suggest mitral regurgitation in the immediate newborn period.

Summary

The effect of placental transfusion upon the portal and atrial pressures of 93 normal newborn infants, ½ to 14 hours of age, was studied. In 34 cases, clamping of the cord was done within 5 to 10 seconds after delivery (EC group); in 59 cases, it was performed after cessation of the umbilical pulsations (LC group).

The portal pressure was always higher than that of the right atrium by an average of 5.6 mm Hg. The portal pressure curves had no venous waves and revealed regular inspiratory rise and expiratory fall. The mean portal pressure of all EC infants was 6.3 ($\pm$0.40 S.E.) mm Hg and that of LC infants 6.5 ($\pm$0.22 S.E.) mm Hg. There was no significant difference in the portal pressures of both groups at the various ages.

The atrial pressures of the LC infants were higher than those of the EC infants during the first 60 minutes following birth ($P<0.001$). Thereafter, the difference between the 2 groups was not significant. The atrial pressures of the LC infants were high before one hour of age but dropped to about half by the second hour and later. In contrast, those of the EC subjects remained about the same in the various age groups.

Left atrial pressure was always greater than that of the right at all ages in both groups. In addition, the left atrial curves showed prominent "v" waves not observed in the right atrial tracings.

The higher atrial pressure observed in the LC infants immediately after birth is attributed to the large placental transfusion allowed these subjects. The pressure drop and its stabilization after the first hour presumably reflects the eff[illegible] accommodation of the augmented volume by the low pressure [illegible] the cardiovascular system

References

1. Arcilla, R. A., Oh, W., Lind, J. and Gessner, I. H.: Pulmonary arterial pressures of newborn infants born with early and late clamping of the cord. *Acta Paediat Scand, 55* 305, 1966.
2. Blankenship, W., Lind, J. and Arcilla, R. A.: Atrial pressures and pulmonary circulation time in the newborn infant. *Acta Paediat Scand,* [illegible] 446, 1965.
3. Bradley, S. E.: The circulation and the liver. *Gastroenterology 44* 403, 1963.
4. Burnard, E. D. and James, L. S.: Atrial pressures and cardiac size in the newborn infant. Relationship with degree of birth asphyxia and size of placental transfusion. *J Pediat, 62* 815, 1963.
5. Cotton, D. and Young, M.: Venous pressure measurements in exchange transfusion. *Arch Dis Child, 40* 323, 1965.
6. Green, H. D.: Circulatory system: Physical principles. In O. Glasser (ed.): *Medical Physics* Vol. 2. Year Book Publishers, Chicago, 1950.
7. Grindlay, J. H., Herrick, J. F. and Mann, F. C.: Measurement of the blood flow of the liver *Amer J Physiol, 132* 489, 1941.
8. Haselhorst, G.: Zum plazentaren Kreislauf unter der Geburt. *Z Geburtsh Gynak, 95* 32, 224 and 400, 1929.
9. Jegier, W., Blankenship, W. and Lind, J.: Venous pressure in the first hour of life and its relationship to placental transfusion. *Acta Paediat Scand, 52* 485, 1963.
10. Krook, H.: Estimation of portal venous pressure by occlusive hepatic vein catheterization. *Scand J Clin Lab Invest, 5* 285, 1953.
11. Margolis, A. J. and Orcutt, R. E.: Pressure in human umbilical vessels in utero. *Amer J Obstet Gynec 80* 573, 1960.
12. Nyberg, R. and W[illegible] in[illegible]fluence of uterine cont[illegible] blood pressure in the umbili[illegible] *Acta Paediat Scand 47* [illegible] 195[illegible]
13. Oh, W., Blankenship, W. and L[illegible] J.: Further study of neonatal blood volume relation to placental transfusion [illegible] in press.
14. P[illegible]ton, A., Reynolds, T. B. and Sherlock, S.: Assessment of portal venous hypertension by catheterization of hepatic vein. *Lancet I* 918, 1953.
15. Peltonen, T. and Hirvonen, L.: The ductus venosus. *Acta Paediat Scand 52* 702, 1963.
16. Schoeler, H.: Fundamental considerations on blood volume: physiologic and clinical aspects. *Amer Heart J 69* 701, 1965.
17. Sherlock, S.: *Diseases of the liver and biliary system,* p. 101 Blackwell Scientific Publ Oxford, 1955.
18. Taylor, W. J. and Myers, J. D.: Occlusive hepatic venous catheterization in the study of the normal liver, cirrhosis of the liver and noncirrhotic portal hypertension. *Circulation, 13* 368, 1956.
19. Usher, R., Shephard, M. and Lind, J.: The blood volume of the newborn infant and placental transfusion. *Acta Paediat Scand 52* 497, 1963.
20. Walker, R. M.: The pathology and treatment of portal hypertension. *Lancet I* 729, 1953.
21. Wallgren, G., Karlberg, P. and Lind, J.: Studies of the circulatory adaptation immediately after birth. *Acta Paediat Scand 49* 843, 1960.
22. Wiedeman, M. P.: Dimensions of blood vessels from distributing artery to collecting vein. *Circulation Res, 12* 375, 1963.

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The Surgical Management of Myelomeningocele with a Preliminary Report of 31 Cases

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In previous communications [7, 29] all cases of spina bifida with herniation of meninges have been classified under the general term spina bifida cystica. This term refers specifically to meningoceles and myelomeningoceles, which are present as a fluid filled sac on the skin surface.

In this report we will discuss the following clinical problems in patients with myelomeningoceles: 1 the selection of cases for operation, 2 the optimal time for operation, 3 the operative procedure.

1 The Selection of Cases for Operation

During the last ten years the management of patients with myelomeningocele has been characterized by a more active surgical attitude. The main reason for this is advances in the surgical control of hydrocephalus and a more skilled rehabilitation [5, 13, 1, 20, 31, 4, 40].

Still, some authors state certain contraindications for operation of myelomeningocele referring to factors as (*a*) the probability of a reasonable normal mental development, (*b*) the extent of the paresis and (*c*) the domestic and economic state of the patient's family [20, 2, 23, 44].

The probability of a reasonable normal mental development. The mental development of a child with myelomeningocele depends mainly upon the existence of an associated hydrocephalus. Because of the complex nature of the intracranial malformation, one hesitates to state that control of the hydrocephalus preserves intellect in all cases but there is a parallelism which indicates that perhaps 3/4 would be normal, if hydrocephalus could be controlled [13].

A dilatated ventricular system is probably present at birth in the majority of cases with myelomeningocele, though obviously without undue skull enlargement [30]. If the hydrocephalus is *arrested* prior to birth or in the early postnatal period, the mental prognosis is considered to be good [7, 18, 19]. If the hydrocephalus is *progressive* the prognosis for life and for reasonable mental development is extremely bad, unless the hydrocephalus is treated early [31]. The thickness of the cerebral mantle in a newborn, however should not be taken as a measure of the degree of cerebral damage and cannot be correlated to the future mental development [29, 44, 45].

The average incidence of active hydrocephalus in the largest series of myelomeningocele is approximately 0 [7 20 34, 41, 44] After the introduction of ventriculo-venous shunts, hydrocephalus can be treated with about 70% success [1]. According to this the probability is about 20% for a newborn with a myelomeningocele to get hydrocephalus, which will not respond to treatment. In comparable clinical series Doran & Guthkelch [7] reported that less than 30 % of the surviving cases of myelomeningoceles with hydrocephalus showed *evidence of mental retardation*. Lorber [31] found that two-thirds of the survivors had a normal intelligence if hydrocephalus was treated at the appropriate time.

As a conclusion we consider that the potential risk for future mental retardation in a newborn with hydrocephalus cannot be regarded as a contraindication for surgery

The extent of the paresis Accurate muscle power assessment of the neonate is often difficult. The paresis present at birth bears no absolute relationship to the further loco-motor development [12, 38] The end result might be influenced by the possibility of central reorganization and peripheral redistribution of available nervous tissue, preserved by early adequate surgery

In the past years there has been considerable controversy as to the management of children born with myelomeningoceles. The *attitude towards surgical treatment* has varied from total neglect of all cases to energetic surgical attack on both the myelomeningocele and the hydrocephalus. This might be exemplified by the following statements:

"No infan l myelomeningocele with paralysis of t egs and sphincters or a *definite hydro p alus should receive any* care except t l him" [43].

The advice that he will almost certainly die or be no good anyway is simply untrue and reflects an ignorance of available modern methods of management [4?]

The care of the unoperated child has always been a matter of great concern to us, we have viewed with distaste the tremendous and frequently unsuccessful *attempts to preserve the sac nd to prevent* infection. We are much happier with the present recommendations and will now feel fully armed to cope with our neurosurgical colleagues who with a disdainful and condescending air have told us which infant will have surgery and which will not Down with the neurosurgeons; let all these lesions be repaired! [11]

Conservative management or delayed surgical treatment can mean nothing but expectance to see if the child will die or not. We fully agree with Sharrard [38], in saying this is rather equivalent to saying to a person who has cancer we won't operate on you but we will wait and see whether you live, and if you do we will know that it is not too bad a cancer and we will be able to operate with success

During the first three years of life the orthopaedic management is mainly prophylactic and is mostly concerned with prevention of contractures where the greatest danger exists in the hips. The bulk of this orthopaedic management at present has to be directed to the correction of deformities, which have arisen from a baby being nursed on its side with its

legs drawn up to its tummy for several years of its life [34]

Those who survive past two years of age usually have sufficient muscle tone to stabilize their hip joints and can be taught to walk with aid of braces

Back pain on attempted movement of the thighs elicited from an unoperated sac may cause flexion contractures of the hip joints and thus prevent walking [33] Thus, we think there is no reason to deny the child the right to live because he has paralysed legs and lacks sphincter tonus. It would be just as unreasonable to discuss the right to existance of a paralytic victim

The domestic and economic state of the patient's family Domestic or economic problems cannot be accepted as contra indicating factors in a civilized community with an advanced standard of health. With energetic team work by different specialists many of these children can be given a future relatively free from handi caps [8 17 1 3. 38, 47]

In conclusion we consider that repair of the sac is indicated in all cases provided the child is in an operable state Any selecting procedure means applied passive euthanasia and has no medical or ethical bearing

2. The Optimal time for Operation

The *primary* aim with surgical intervention on myelomeningoceles is to prevent ascending infection. If left untreated there is a great risk of meningitis which is the usual cause of death during the first weeks of life [3.] It is exceptional that the sac is totally covered by skin [42]. In such cases there is no urgency for surgical management, provided the skin is sound and intact On the other hand there is a strong liability for slowly increasing paralysis to develop as the child grows [38] In those cases we recommend surgical excision of the lesion during the first month of life.

Ascending infection involving the spinal and cranial subarachnoid space may also increase the incidence and severity of the hydrocephalus. The most severe pyogenic infections are associated with severe parenchymal damage [28].

An infection on the surface of the sac can spread not only by a rupture of the sac but also through fissures and ulcerations of the thin membranes. In most cases of myelomeningoceles, the central canal of the cord opens free on the surface offering a direct communication between the outer environments and the cord (Figs. 1 2). Early operation seems necessary to prevent infection for once present, it is difficult to control [32]. Some authors still advocate waiting for some months before repair of the defect [18 34], because the sac is then epithelialized and the danger of introducing an infection within the theca is supposed to be less.

The *ultimate* goal of the operation is to prevent further neurological sequelae. Dehydration and infection of the neural plate and the nerve roots may lead to destruction of neurons in a few hours [28]. Stretching of the nerve roots as the sac distends after birth may be a factor responsible for deterioration of the neurological status. Secondary scar tissue formation often causes progressive impairment of motor and sensory functions, leading to severe neurological deficit [4]. Early closure of the defect, preferably on the first day of life can usually prevent

this additional damage [31 40] and even result in improvement of muscle function [39]. Moreover we consider that immediate neonatal surgery is less hazardous because the neural structures are better visible and easier to free before epithelization and granulation have distorted the involved tissues.

One of the main objections to early operation of the myelomeningocele was that the removal of the sac would accelerate or even elicit hydrocephalus [6, 37]. This theory has been contradicted by others [9 14 *6, *]. It is possible that removal of the sac in some cases may accelerate the hydrocephalus [7], but the correlation is not clear and this hypothetical risk should not influence the decision regarding early closure of the sac [28]

Some authors prefer a primary attack on a hydrocephalus before embarking on back surgery [16, 4_]. They suggest that adequate decompression of the hydrocephalus may so flatten the back mass that the repair is no longer required, and also that postoperative aggravation of hydrocephalus is averted if an Arnold-Chiari malformation should precipitate into acute medullary impaction or should an absorptive bloc occur from blood or infection.

In most untreated cases of myelomeningocele the sac slowly enlarges and sudden rupture with escape of large quantities of cerebrospinal fluid may cause death from convulsions [10]. There are also nursing problems associated with a thin-walled sac. It requires sterile dressings and will be a constant burden upon the parents as a source of great alarm and anxiety

We believe it is advantageous that the spinal defect is closed and healed before an accompanying hydrocephalus is treated

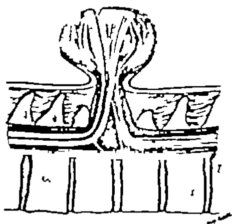


Fig. 1 Sagittal view demonstrating distribution of nervous elements. The central canal runs through the sac cavity enclosed in nervous tissue to its opening on the anterior part of the neural plate.

This is usually possible if the sac is closed in the immediate neonatal period. If a severe progressing hydrocephalus is present at birth, we usually repair the myelomeningocele and control the hydrocephalus by frequent ventricular taps or continuous ventricular drainage When the spinal wound has healed and the ventricular fluid is sterile we perform a shunt operation.

In conclusion early operation of the myelomeningocele probably decreases the mortality and morbidity An infant stands the surgical and anesthesiological procedures quite well in the immediate neonatal period Therefore we prefer to operate during the first day of life as soon as possible after delivery

3 The Operative Procedure

Many children, born with myelomeningocele have a hopeless prognosis at birth but some have the potential for reasonably



Fig. 2. A soft catheter is introduced into the central canal through the preformed opening on the neural plate.

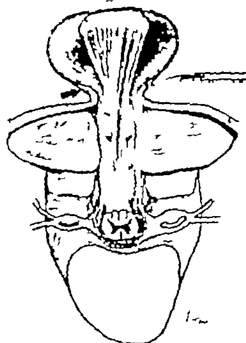


Fig. 3. Frontal view of myelomeningocele showing the level of skin incision.

satisfactory existence under optimum circumstances that is surgeons experienced with the care of infants, the operative technique and the many factors of the problem.

Although we stress the importance of early operation we believe that it is better to delay the operation some hours for transport than to have it performed by an unexperienced surgeon, working with a strange team in a strange surrounding and with an inadequate equipment [30].

The surgical principles are simple: (1) Good skin covering is absolutely necessary for optimal result. ( ) All functioning neural tissue should be saved.

The sac should be incised in the border between the skin and the thin membranes (Fig. 3). Even if the skin looks *thin* and red it is practically always viable and usually resumes normal colour when the

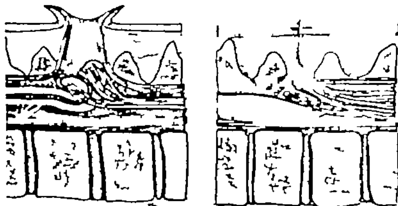


Fig. 4. Sagittal view showing ( ) oozing of cerebrospinal fluid from the central anal and adhesions between the cord and arachnoid membrane t the neck of the sac (b) the myelomeningocele after surgical repair The subarachnoid adhesions have been se ered permitting cerebrospinal fluid to escape int the spinal subarachnoid space

sac is emptied with release of the tension.

The anatomy of the neural elements can be inspected, when the sac has been opened (Figs. 1) The neural plate should be freed cautiously from the surrounding zone of thin membranes If skin covers the top of the sac close to the neural plate all epithelium should be excised to prevent the development of epidermoid cysts [46]. The spinal roots usually run freely in the cavity but some times single nerve roots have to be dissected free from the sac. Traction on medulla or roots should be avoided. They are just as sensible as normal nervous structures [.5].

There are often dense adhesions between the dysplastic cord and the meninges at the neck of the sac. These adhesions have to be severed in order to prevent traction during the later growth of the vertebral column. Furthermore, a continuity could thus be established between the central canal and the spinal subarachnoid space This

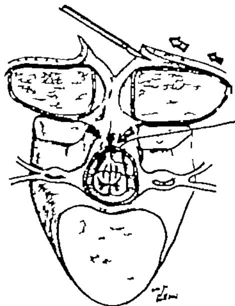


Fig 5. Frontal view showing closure of the dura and undermining of skin by lateral subcutaneous dissection.

Fig 6. F ont l [illegible] z l pl [illegible] m [illegible] w h

comn [illegible]) l t) ut format n of loc [illegible] (l fi ula and even po- bl l [illegible] ity of an a companying hydrocephalu (l c 4) (i tl Kelch (15) report that early co v ring of the bare area by f ee sk n grafts as suggested by Lauren e (8) an fail because of leakage of cerebrospinal fluid from the central anal Laminectomy on the rostral vertebral arch in order to facilitate this dissection i hardly ev r needed.

The dura should be incised at the junction of thin skin and normal skin and should be freed by blunt dissection so far that the extradural part of the roots are exposed Generally there is sufficient dura to construct a meningeal tube which will enclose the neural plate and the roots without tension (Fig 5) If the amount of available dura is unsufficient for a closure without strangulation of the neural elements, it is preferable to leave it open A covering duplication using the lumbo-acral fascia is made in order to strengthen he repair (Fig 6) The skin should be sutured without undue tension. The preserved skin from the base of the sac is used and the approximation facilitated by xtremely wide dissections of the skin on each side of the lesion (Fig 5) Thus it is very seldom necessary to use skin flaps for closure

Case Material

During the last three years we hav been ble to treat patients with myelomeningo-ele using the principles stated above: no selection of cases and as early surgery as possible. The studied group consists of pa ients referred for treatment during the years 1963 and 1964, which enders an observation ime of at least one year It has not been our ntention to estimate the mental and physical levelopment but t study the mortality te although the material is rather small.

The studied group consists of 31 patients. One child was not subjected to surgery because of inoperability due to pulmonary failure to which he succumbed on the second lay after birth. 30 patients were operated upon with repair of the spinal defect The time for operation is illustrated in Fig. 7 One patient got a se ere wound infection and died of a purulent meningitis five days after deli ery Hydrocephalus, where surgical inter ention was indicated, developed in 23 (79 %) of the remaining 29 patients. Ventriculo-venous shunts were used in all cases and the time for this procedure is illustrated in Fig 8.

Hydrocephalus was obvious at birth in three cases. In the other 20 it was rev ealed by serial examinations—e.g. head circumference fontanel tension, echo-encephalography sutural separation. In most cases air studies were performed to confirm the diagnosis.

N o of the patients in the hydrocephalic group have died due to progressing hydrocephalus and/or complications from the shunts. Hydrocephalus is clinically com-

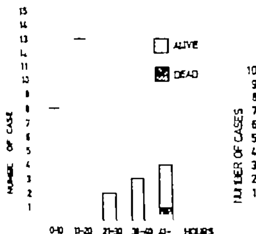


Fig. 7 Age at operation of myelomeningocele.

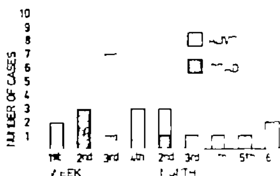


Fig. 8. Age at operation of hydrocephalus.

pensated in the remaining 18 patients. Hydrocephalus did not develop in six patients and they are all in good condition. The whole course is illustrated in Fig. 9.

Thus, 24 children of 31 born with myelomeningocele have survived one year or more which suggests a survi al of 8 .

One year or more after operation the head circumference was normal for age in ten of the 18 patients with adequately functioning entriculo-atrial shunts. In f pa tients it was less than 3 cm above the normal and in three patients more than 3 cm above the normal range. Clinical assessment f the mental status is very indefinite but most f the children ppear to behave normal for age.

Discussion

The survival rate (78 %) is rather high in this series. We consider this due to early repair of the spinal defect which has almost abolished the risk of meningitis. Furthermore it is important with an energetic treatment of the accompanying hydrocephalus to lessen the mortality rate It seems probable that the time for surgical intervention to control hydrocephalus is a significant factor in relation to subsequent mental status. The hydrocephalic children should be subjected to frequent examinations which gives possibility to early handling of shunt complications In the effort to obtain compensation of the hydrocephalus, it should, if necessary be attacked by other methods [1].

Currently we do very extensive x ray studies in order to visualize the cerebrospinal fluid pathways In this way it seems possible to find criteria where a direct surgical attack on the malformations in the posterior fossa is indicated [ ]. These stu-

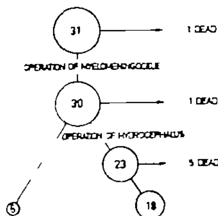


Fig 9 Fate of 31 children born with myelomeningocele.

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CASE REPORT

Fatal BCG Infection in an Infant with Congenital, Lymphocytopenic Agammaglobulinemia

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In relation to the tremendous number of BCG vaccinations performed the world over (probably more than one hundred millions) noticeably few serious complications seem to have occurred. To the best of our knowledge only thirteen fatalities in connection with BCG vaccination have been reported [1 3 5 8 9 10 11 18 20 22, 23 26]. Two patients were 7 and 19 years, respectively at vaccination and died two and five years later [20 23] The others were infants who had been vaccinated in the neonatal period. Five of the cases are reported from the Scandinavian countries.

In this report a new case of fatal BCG infection is described in which the clinical course and the laboratory and pathologico-anatomic findings also disclosed a congenital agammaglobulinemia of the lymphocytopenic type.

Case Report

U G 640321 A boy the only child of healthy non-consanguineous parents, with a birth weight of 3000 g was BCG vaccinated when newborn without local complications. H remained well and developed normally up t the age of six months when he was hospitalized for bronchopneumonia and diarrhea. Stool culture disclosed pathogenic E coli, serotype O 128 B 12 and the boy was treated with broad spectrum antibiotics after this, stool cultures became negative. However the diarrhea continued and radiograms f the chest showed progress of the pneumonic consolidations. Thrush appeared in the oral cavity and became quite exten sive. Mycotic infection of the lungs was then suspected and t eatment with nystatin was instituted When this seemingly had no ffect the patient was referred to the pediat ric clinic for observation

On admission, about two months after the beginning of the illness, the boy was in a fairly poor condition. His weight was 6800 g, the subcutaneous fat was reduced in amount and there was slight to moderate tachypnea and dyspnea. Rales were heard over both lung fields. The li er and spleen were not enlarged. There were no lymph nodes pal pable ither in the left groin or elsewhere and except a barely visible scar on the left thigh there were no signs of local reaction to the BCG accination. The oral cavity and the pharynx were normal and without thrush on this occasion; a special note on the p pearance of the tonsils is lacking. On oesophagoscopy and bronchoscopy the mucous membranes had a normal appearance and there were only insignificant amounts of

mucopurulent secretion. There was no fever and the E.S.R. was mm/1 hr The Mantoux reaction was negative (1 100) as in the central county hospital one month earlier. Chest radiogram showed bilateral partial consolidations of the pulmonary parenchyma. Nasopharyngeal swabs and blood and stool cultures yielded no growth of pathogenic bacteria or fungi. Later acid fast bacteria were found in the gastric la age fluid when cult ted on Lowenstein-Jensen medium, but this was revealed after the patient had died.

Treatment with nystatin wa continued and, in addition, sulphadiazine was given. Within two weeks radiography showed decrease f the pulmonary consolidations and this improvement continued. However the diarrhea became worse with stool amounts as high as 700 à 1000 g daily In addition, a considerable number of subcutaneous nodules, up to the size of a pea, with a firm consistency ppeared on various parts of the body Biopsy revealed a granulomatous tissue with epithelioid cells and a few lymphocytes. Ziehl-Neelsen staining brought out innumerable red-stained, slender dumb-bell shaped rods. These probably were mycobacteria but unfortunately were interpreted as possibly being mycotic mycelia (generalized fungus infection was strongly suspected at this time); no culture was made from this biopsy material.

On paper electrophoresis no gamma globulin was found (t tal serum protein 6.0 g/100 ml, gamma globulin 0 g/100 ml). Immunoelectrophoresis showed complete absence of gamma A and gamma M globulins and only insignificant amounts of gamma G (total protein 5.8 g/100 ml, albumin 3.8, alpha$_1$ 0.25, alpha$_2$ 0.87 beta 0.55, beta$_2$ 0.18 and gamma 0.09 g/100 ml) Both parents had normal serum protein levels and all immunoglobulins within normal range The patient belonged to blood group A Rh − as did both parents, but he had no isohemagglutinins. The t tal whit cell count aried between 16,000 and 000 per mm^3 except in the final stage when there was leucopenia. However during the whole course there was a constant moderate lymphocytopenia with total counts between 3500 and 900 per mm (Fig 1). There was neither anemia nor thrombocytopenia. Bone marrow smears showed a normal erythro-, myelo- and thrombopoesis while the lymphocytes although of normal appearance were much reduced in number 1.5 of 1000 counted cells. The plasma cells were very scarce 0.-%, but otherwise normal. The reticular elements were increased.—No lymph nodes were available for biopsy

Due to the lack of gamma globulin, substitution was given. The diarrhea persisted, however and malabsorption became the predominant problem. Only 74% of ingested fat was found to be absorbed. Because of the poor condition of the patient no further studies on the intestinal function were performed.

At this stage a transplantation of fetal thymus to the patient was planned. As a first step a piece of skin was transferred from the father However the boy died shortly thereafter and the fate of the transplant could be followed for only 16 days. During this time no homograft rejection took place

During the last 2 3 weeks of lif the patient had fever tachypnea and increased E.S.R In spite of intensiv treatment with sulphadiazine, penicillin and streptomycin, the latter drug, however for two days only his condition rapidly deteriorated. Peripheral edema, hemorrhagic diathesis and laboratory signs of bone marrow depletion appeared and the boy died at the age of nine months.

Autopsy findings

Macroscopic examination A boy weighing 6,?20 g and measuring 68 cm without external malformations. On the trunk numerous petecchiae and occasional ecchymoses. In the subcutis disseminated p to pea-sized tumor-like masses having a yellowish white cut surface These were especially abundant along the thighs. The heart weighed 60 g and exhibited a slight dilation of the right entricle. Pericardium myocar



Fig. 1 Total peripheral blood lymphocyte count during the course of illness.

dium and endocardium normal. No septal defects. *Ductus arteriosus* closed. The pleural membranes were dull and thinly coated with fibrin, no pleural fluid. The consistency of the lungs was firm and parts of the lungs had a dark red cut surface from which could be expressed a turbid material. The liver weighed 450 g and was of normal consistency and color. Biliary ducts, gall bladder and pancreas normal. The kidneys weighed 100 g together and were of normal shape and appearance. The adrenals normal. The spleen weighed 60 g and was permeated by up to pea-sized yellowish white foci, partly confluent. Para-aortically in the abdomen several up to twice almond-sized lymph nodes with grey and yellow mottled cut surfaces. The thymus weighed 1.5 g. The brain weighed 450 g and the meninges and the parenchyma displayed no focal lesions.

Microscopic examination. The subcutis was the site of scattered necrotic foci in whose neighbourhood there were a few epithelioid cells and lymphocytes. Ziehl Neelsen stained sections exhibited an abundance of acid fast rods. The lungs showed extensive haemorrhages and non-acid-fast bacteria in abundance, indeed veritable colonies. There was a sparse cellular inflammatory reaction. Scattered epithelioid cell granulomata with acid fast rods were present. No *Pneumocystis carinii*.

The liver was the site of scattered, small necrotic foci with a few pale epithelioid cells in the neighbourhood and plenty of acid fast rods. Moreover the liver exhibited the picture of blood stasis and minor fatty degeneration. Small necrotic foci with plenty of acid fast rods were also seen in the adrenal cortex as well as in the medulla. The normal splenic structure was almost completely destroyed, containing necrotic foci of varying size with a few epithelioid cells and giant cells in the periphery. No cuffing by lymphocytes and no germinal centers. Para-aortic lymph nodes were totally and subtotally destroyed by large necrotic and necrobiotic foci. No tubercles present. Myriads of acid fast rods were present in both spleen and lymph nodes.

The thymus had widened interlobular septa and small, poorly developed lobules, no differentiation into cortex and medulla and no Hassal's corpuscles, (Fig. 2). Within the lobules there were a few widely scattered small lymphocytes. In the thymus necrotic foci of varying sizes with a few epithelioid cells and plenty of acid fast rods (Fig. 3). No tuberculous changes in the pancreas and the kidneys. The brain tissue lacked focal lesions.



Fig. 2. Thymus with poorly developed lobules and no differentiation into cortex and medulla and no Hassall corpuscles. In lobule a necrotic focus. (van Gieson, ×3.)

but the pia exhibited signs of mild, chronic, unspecified leptomeningitis.

Bacteriological findings

Gastric lavage fluid and various autopsy specimens were examined in regard to mycobacteria. Cultivation on Lowenstein Jensen medium of samples of the former showed 3–9 small colonies of acid-fast rods resembling tubercle bacteria in each of the 4 inoculated bottles after 6 weeks incubation. No colonies were observed at the preliminary 2 and 4 weeks readings. The guinea pig tests were negative.

The autopsy specimens comprised skin spleen, kidney pleura peritoneum blood cerebrospinal fluid and pericardial fluid. Direct microscopy of Hallberg stained smears from some samples showed abundant acid fast rods, particularly the sample of spleen contained copious amounts of acid fast material. All of the samples were tested on Lowenstein-Jensen medium and guinea pigs. All of the cultivations evidenced growth of mycobacteria resembling tubercle bacteria, while all of the guinea pig tests were negative.

The isolates from the gastric lavage fluid, skin and spleen samples were used for typing.

Their ability to grow at 37°C and 22°C was tested as well as their sensitivity to isoniazid and furan 2-carbonic acid hydrazide [7], niacin production nicotinamidase activity and transhydrogenase activity [19]. A summary of the results is found in Table I.

The typing results show a pattern which indicates that the three isolates tested behaved as BCG.

Discussion

From the autopsy findings it is evident that the patient had a widespread tuberculous disease with a pathologico-anatomic picture corresponding to that which has been described in cases of generalized BCG infection reported previously. The bacteriologic investigation confirmed that the actual changes were caused by mycobacteria, most probably BCG. Since *Pneumocystis carinii* pneumonia was present. In addition, in two of the previous cases [5



Fig. 2. Thymus with large necrotic area to the left and few lymphocytes and epithelioid cells in the periphery of the necrosis. (van Gieson, ×23.)

10] signs of this infection were sought but not found.

The vaccine which had been used in the present case was a lyophilized vaccine supplied by the BCG laboratory in Gothenburg. The batch number of the vaccine was not registered by the vaccinator but to the best of our knowledge no complications have occurred in the other ten individuals which were vaccinated on the same occasion and with the same batch of vaccine. Consequently there is no reason to assume an increased virulence of the BCG vaccine used in the present case as little as in the cases reported previously. In some of the papers this fact was parti-

TABLE 1. *Results of typing tests of the mycobacterial isolates*

Source of isolated organism	Sensitivity to		Growth on L-J medium		Pathogenicity guinea pig	Niacin test	Nicotin amidase activity	Trans-hydrogenase activity
	INH	PtH	37 C	22 C				
Gastric lavage fluid	+	+	+	−	−	−	−	−
Spleen	+	+		−	−	−		−
Skin	+	+	+	−	−	−		−
Key								
M. tuberculosis	±		+		+	+	+	+
	±	−	+		±		+	
M. bovis	±	+	−		−	−	−	+
	±				±	−	−	+
BCG	±	−	+		−			−
	±	−	+		−	−		
Unclassified mycobacteria	−	−	±	±	−	−	±	+

cularly pointed out [10 11 20 23], actually such changes of the BCG vaccine have never been proven. The only explanation, then, for the fatal course of the vaccination in these patients is that they had a lowered resistance to BCG

In the first papers concerning generalized BCG infection the authors in question were unable to explain satisfactorily the cause of the decreased resistance to vaccination. In 1958 however Ramon-Guerra *et al* [2.] drew attention to a probable connection between atypical becegeitis" and agammaglobulinemia. In later more thoroughly studied cases an immunologic defect is also shown to have been present the nature of which now seems to be quite well defined.

In the present case the clinical course and the laboratory investigations, especially the immuno-electrophoresis which revealed complete absence of gamma A and gamma M and insignificant amounts of gamma G indicated a congenital agammaglobulinemia. Obviously this was not of the hereditary sex linked type which was first described by Bruton [6] nor of the transient form which is said to be indistinguishable from the former at this early age. The constant moderate lymphocytopenia, the inability to develop delayed allergy against tuberculin and above all, the pathologico-anatomic findings of a small thymus without differentiation into cortex and medulla and without Hasall s corpuscles were in keeping with a congenital agammaglobulinemia of the lymphocytopenic or 'Swiss type [1., 13 14 16, 4]

Among the cases of f tal BCG vaccination which ha e been reported previously complete serum immune globulin studies, including immunoelectrophoresis, were made in only two, namely in the cases reported by Bouton *et al* [5] and by Villemin [26] In both agammaglobulinemia was shown to be present. Autopsy revealed besides disseminated BCG infection almost complete absence of lymphoid tissue indicating that the agammaglobulinemia was of the lymphocytopenic type In the case described by Falkmer *et al* [10] no gamma globulin studies were performed but the pathologico-anatomic description of the thymus tallies perfectly well with a lymphocytopenic agammaglobulinemia. In addition, a younger brother of this patient is known to have had agammaglobulinemia verified by immuno-electrophoresis and when examined postmortem showed defective development of the thymus and other lymphoid tissues [4] Most probably therefore, lymphocytopenic agammaglobulinemia was present in the elder brother too

Thus, in four cases at least, including the present one agammaglobulinemia of the lymphocytopenic type was indubitably present. In the other cases immunoglobulin studies were either incomplete or not performed and the autopsy records do not give sufficient information to establish a diagnosis of lymphoid hypoplasia. However in view of some of the clinical and laboratory data available viz., the presence of generalized moniliasis, persistent diarrhea and/or interstitial pneumonia in the patient or in one or more siblings; a persistently negative tuberculin reaction, and a differential whit blood cell count showing lymphocytopenia one may conclude that lymphocytopenic agammaglobulinemia (or possibly alymphocytosis without agammaglobulinemia, a condition

which has been described recently by Nezelof et al [21]) was probably present in the cases of Arutia et al. [3] and Gardborg et al [11] and the two cases of Ramon Guerra et al. [22]. On the other hand, in the cases reported by Hollström & Hård [18] Araya et al [1] Chiari & Zischka [8] and Dzieniszewska Klepacka & Levicki [9] the information available is not sufficient to allow any conclusions regarding a possible immunologic defect For particulars, the reader is referred to the treatise of Villemin [26] where the literature is thoroughly reviewed.

The cases of Meyer & Jensen [20] and Thrap-Meyer et al [23] differ in many respects from the ones already mentioned. Whereas the latter all died in early infancy after a short illness the forementioned patients were 7 and 10 years, respectively at onset, and their disease had a more protracted course dominated by bone lesions, abscess formation and cachexia and lasting for two and five years, respectively The tuberculin reaction was slightly positive in Meyer & Jensen's case and at first positive and later negative in the case of Thrap-Meyer et al. contrary to the persistently negative tuberculin reaction in all the other cases. Finally the gamma globulin level was increased in Thrap-Meyer's case whereas it was not determined in the case of Meyer & Jensen. Whatever the cause of the decreased resistance to BCG vaccination may have been in these two cases agammaglobulinemia was obviously not present. Perhaps they come closer to the cases of nonfatal bacterial metastasis following BCG vaccination which have been reported by among others Wallerström & Enell [25] who were unable to find an indubitable immunologic defect in their patients.

It is highly probable that the fatal course of the BCG vaccination was due to the immunologic defect which apparently was present in most cases. It is true that patients with agammaglobulinemia may be able to handle a tuberculous infection, and not a few cases have been reported where BCG infection has had an uneventful course and resulted in a positive tuberculin reaction [15] These patients, however may have had agammaglobulinemia of the nonlymphocytopenic type in which the cellular defense mechanism is unimpaired. The present case, on the other hand, and most of the previously reported cases of fatal BCG infection more or less evidently had the lymphocytopenic form of agammaglobulinemia. Obviously individuals who have a defect of both the cellular and humoral defense mechanism deal very poorly with tuberculosis and BCG vaccination.

From the viewpoint of resistance to tuberculosis it may be of interest that there seems to exist a third type of immunologic defect where only the cellular and not the humoral defense mechanism is affected. The observation made by Nezelof et al. [21] was already mentioned. Patients with Hodgkin disease have an immunologic defect which might be of a similar type [14]. They do not have agammaglobulinemia but often lack delayed hypersensitivity reactions and have defects to transplantation immunity Earlier investigations showed an increased incidence of tuberculosis in patients with Hodgkin's disease compared to the general population [13]. Data are lacking, however regarding possibly lowered resistance to BCG vaccination in such individuals.

The pathologico-anatomic picture in generalized BCG infection strongly re-

sembles the one seen in so-called non reactive generalized tuberculosis described by among others Arends [?] Both of these forms of tuberculous disease are characterized by extensive necrosis very small amounts of tuberculous granuloma tissue and an extraordinary abundance of tubercle bacteria. The nonreactive generalized tuberculosis has been described in conjunction with leukemia, agranulocytosis and panmyelophthisis, which may indicate some defect of the cellular defence mechanism also in these diseases bringing about an inability of the body to wall off the tuberculous infection.

In this country most BCG vaccinations are performed in the neonatal period. Only in the presence of a positive family history would it be possible, at this early age, to suspect agammaglobulinemia and by refraining from vaccination, to avoid fatalities like the one under discussion. Moreover in the case reported by Bonnevier *et al.* [4] this precaution did not preserve the infant from early death following a septic infection. In fact, this type of immunologic defect has, to the best of our knowledge proved fatal in every case

In the present case the diagnosis of disseminated BCG infection was not established before death and, consequently no specific treatment was instituted Such treatment, however was apparently not successful in those of the previous cases (3 5 18, 22, 23 26) where the diagnosis was made *intra vitam*

Pooled gamma globulin was, of course given to the patient as substitution but evidently this treatment did not benefit him any more than it did the patients of Bouton *et al* [5] and Villemin [26]

Considering the role played by the normal thymus in the immune defense of the body [12, 13, 16] it is reasonable to try to transplant thymic tissue to patients with lymphocytopenic agammaglobulinemia. Although homografts are generally well tolerated by these individuals [13, 16], as far as we know such attempts have not yet succeeded. Hitzig *et al.* [17], using the combination of fetal thymus implantation and intravenous infusion of liver cells from the same fetus, observed a rise of the peripheral blood lymphocytes during the treatment but their patient ultimately died. These authors, however suggest that further trials along these lines should be made.

Summary

A case is described of fatal disseminated BCG infection in an infant with lymphocytopenic agammaglobulinemia. The report includes clinical, pathologico-anatomic and bacteriologic findings. A short review of the cases reported in the literature is given. The relation of the BCG infection to the immunologic defect is discussed as well as possible prophylaxis and therapy

References

1 Ara A, Garties and Gutman 1958. Quoted by Arutia, A. *et al* [3].

2 Arends, A Blood disease and so-called generalized non reactive tuberculosis *Acta Med Scand*, 136 417 1950.

3 Aruttia A Moreno, L G acky, C and Monterro R Caso fatal de generalization de B.C G *Rev Chile Pediat* 31 76, 1960.

4 Bonnevier, J O Killander, J Olding, L and Vahlquist B., Congenital agammaglobulinaemia in the brother of boy who died of generalized BCG infection. *Acta Paediat Scand* 11 35, 1944.

5 Bouton J Mainwaring, D. and Sutherland, R. W B C G dissemination in congenital hypogammaglobulinaemia. *Brit Med J* I 1512, 1962

6. BRUTON O. C.: Agammaglobulinemia. *Pediatrics, 9* 22, 1952.
7 BÖNICKE, R.: Über die tuberkulostatische Wirksamkeit pentaheterocyclischer Carbonsäurehydrazide. *Z Hyg I fektionskr 140* 263, 1954.
8. CHIARI, H. and ZISCHKA, W Zur pathologischen Anatomie und Bacteriologie der BCG-Impfung. *Zbl Allg Path, 100* 105, 1959.
9 DZIEWISZEWSKA KLEPACKA, A. L. and LEWICKI, Z. 1958. Quoted by *Villemin* p. 19 [26].
10. FALKMER, S., LIND A. and PLOMAN L.: Fatal tuberculosis from BCG vaccination. *Acta Paediat Scand, 44* 219, 1955.
11 GARDBORG, O., IVERSEN, O H., TORBJESEN, B J and HESSELBERG L: Generalized BCG infection with fatal course in an infant. *Acta Paediat Scand, 51* 293, 1962.
12. GITLIN D. and CRAIG J Thymic alymphoplasia and lymphocytic hypoplasia and their relation to infection. *Pediatrics, 32* 517 1963.
13. GITLIN D., ROSEN, F S. and JANEWAY C. A.: Delayed hypersensitivity and homograft survival in a child with thymic alymphoplasia. *Pediatrics, 33* 711, 1964.
14. GLANZMANN E. and RINIKER, P.: Essentielle Lymphocytophthise Ein neues Krankheitsbild aus der Säuglingspathologie. *Ann Paediat, 175* 1 1950.
15. GOOD R. A., KELLY W D ROTSTEIN J and VARCO, R. L. Immunological deficiency diseases. *Progr Allergy 6* 187 1962.
16. GOOD, R. A., PETERSON R. D A. and GABRIELSEN A. E.: The thymus; current concepts. *Postgrad Med, 36* 505, 1964.
17 HITZIG, W H., KAY H. E. M. and COTTIER, H.: Familial lymphopenia with agammaglobulinemia. An attempt at treatment by implantation of foetal thymus. *Lancet, II* 151 1965.
18. HOLLSTRÖM, E. and HÅRD S.: A fatality from BCG vaccination. *Acta Dermato-Venereol, 33* 159, 1953.
19. JUHLIN, L: Methods for the grouping and typing of mycobacteria. *Acta Path Microbiol Scand, 50. 2* 177 1960
20. MEYER, J and JENNEY K. A.: A fatal case of tuberculosis produced by BCG *Amer Rev Tuberc, 70* 402, 1954.
21 NEZELOF C., MELLE JAMMET M. L., LORTHOLARY P LABRUNE, B. and LAMY M.: L'hypoplasie héréditaire du thymus. Sa place et sa responsabilité dans une observation d aplasie lymphocytaire normoplasmocytaire et normo-globulinémique du nourrisson. *Arch Franc Pediat, 21* 897 1964.
22. RAMOS-GUERRA, A. U QUEIROLO, C. A. and TENORIO, N Síndrome de becegeitis grave, moniliasis extensiva hipogamma globulinemia familiar *Arch Pediat Urug 29* 618, 1958.
23. TRIMAP MEYER, H., OHDING, P., HEIMSBERG, I. and WAALER, E.: Generalized BCG infection in man. *Acta Tuberc Scand. 29* 173, 1954.
24. TOBLER, R. and COTTIER, H.: Familiäre Lymphopenie mit Agammaglobulinämie und schwerer Moniliasis. *Hel Paediat Acta, 13.* 313, 1958
25. WALLGRENSTRÖM, A. and KNELL, H.: Bacterial metastasis following BCG vaccination. *Acta Paediat Scand, 36* 317 1948.
26. VILLEMIN, P *Alymphocytose et risque vaccinal.* Thesis, Paris 1963.

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PROCEEDINGS OF PEDIATRIC SOCIETIES

The Norwegian Pediatric Society

Meeting Jan. 25, 1965

H. Andersen (Copenhagen) Hypothyreosis in children

Axel Søbren. Feminizing Adrenal Hyperplasia

A 3-year-old boy had been treated for the salt losing type of adrenal hyperplasia since the neonatal period without any serious complications. His genitals were abnormal: Short penis, half separated scrotal folds without palpable gonads; the urethral opening and a vaginal recess forming a sinus urogenitalis. Both testes were in the inguinal canal. He had a normal male karyotype. The findings point to a defect of 3-beta-ol dehydrogenase. This defect leads to insufficient masculinization of the genitals (or feminization). The present case clearly demonstrates this and will be published later in detail.

Meeting March 1* 1965

Odd Garberg: Enteropathogenic *Escherichia Coli* Gastroenteritis

Although enteric disease associated with enteropathogenic *E coli (EEC)* has been recognized for many years, very little attention has been accorded to these infections in Norway. During a period of 12 months, 75 strains of EEC were isolated from 62 infants and children hospitalized at the children department, Sentralsykehuset Trondheim.

Thirty-one of the patients were in the age group 0 to ½ year and 58 were under 2 years of age. The most common serotype was O 86, but infections due to O 26, O 55, O 114, O 124, and O 125 were also observed. Thirty four of the infected patients suffered from gastroenteritis of varying severity whereas no intestinal symptoms were demonstrable in 28 cases. During January and February there was no case of EEC—otherwise EEC appeared to occur all through the year. Fiv patients admitted because of severe dystrophy harboured EEC and, on eradication of the infecting strain, a marked clinical improvement was observed. Two of the patients died. Both had serious underlying disorders and neither suffered from gastroenteritis. The EEC infection was judged as of no importance as a cause of death.

Various drug treatments were tried in the present study—mostly colimycin but also nalidixic acid (negram), ampicillin and chloramphenicol. In several cases prophylactic use of colimycin seemed an effect mea sure to prevent cross infection, but as EEC strains have a marked tendency to develop resistance to antibiotics, it can hardly be recommended.

The outbreaks of EEC gastroenteritis in recent years have not been severe and the mortality rates reported have been less than 5 per cent. However it should be remembered that EEC enteritis may be very lethal disease with a mortality rate as high as 50 per cent.

Arne Njå. Treatment of Inguinal Hernia in Infants

In later years, the former conservative treatment of inguinal hernia has been replaced by early surgical intervention.

To get an impression of the results obtained using the conservative bandaging method, the author has for some years been collecting a material of children treated in this way. The material originally comprised 21 children, one of whom was omitted as the therapeutic schedule had not been followed. In all cases, treatment was instituted at 1 to 3 months of age. Only in one case did it prove difficult to keep the hernia from protruding—the exception was a premature boy with a birth weight of 1730 g who was operated at 2½ months of age. In the remaining 19 the treatment was carried through according to plan. Thirteen of these have had no relapse this far with a period of observation from 1 to 7 years, whereas 6 have relapsed and have been operated at the age of 1 to 7 years. No instance of incarceration has occurred. Thus, until today 13 have avoided operation and those who have been operated have gained a postponement till long after infancy. The conservative treatment does not seem to have involved any risk or inconvenience to the children.

In the author's opinion, operation as soon as possible after the diagnosis has been made should be the treatment of choice also in infants. However, in cases where immediate operation for one reason or another seems unadvisable, the oldfashioned bandaging will still serve a useful purpose.

Meeting April 23 1965

Round Table Conference on Congenital Dysplasia of the Hip

Participants: *K. Palmén* (Falköping), *I. Ah k*, *E. Bull Hansen*, *A. Njå* and *D. Aarskog*

Meeting May 21-22, 1965

S. Hesterun. de Toni-Debré-Fanconi Syndrome with Cystinosis. To be published elsewhere.

Roar Funder. Plastic Surgery in Some Congenital Malformations

A summary description of the more common congenital anomalies treated by plastic surgery was given. Patients were demonstrated and slides of cases with cleft lip and palate, hypospadias, syndactylism, ear anomalies, and hemangiomas were presented. Account was given of some therapeutic principles and the date of admission to the hospital stated in each separate case.

Roald Hiartk. Primary Arteritis in Children

"Pulseless Disease" is a term applied to a variety of syndromes which have in common an absence of pulsation in areas supplied by the main arteries. Originally the term was used for an obliterative arteritis of the aortic arch, fairly frequent in the Eastern part of the world and mostly occurring in young women. The condition is very rare in children. The stenosing process is not known—the latest hypothesis is that some autoimmune mechanism is involved. The symptoms are dependent upon the site of the principal vascular occlusions and the adequacy of the collateral circulation. In addition to the pulseless areas, hypertension may be a characteristic feature—the hypertension varying according to degree of occlusion of the artery involved. The elevated blood pressure will lead to secondary heart failure. Angiography is of prime importance for the diagnosis as it will reveal the site of, and the degree of the occlusion. The prognosis is poor and no really effective therapy has yet been found. Six cases (5 female) are reported, all

from 3 to 1 years of age. Characteristic findings are demonstrated and the cases briefly discussed.

Harald M Srendsen Isoimmune Neonatal Thromboeytopenic Purpura

There is good reason to believe that neonatal thrombocyt penic purpura in some cases is caused by an isoimmune mechanism involving maternal-fetal incompatibility of platelet antigens, analogous to incompatibility of erythrocyte antigens in erythroblastosis foetalis. The frequency seems to be one case in 10 000 births. The disease has previously not been described from Norway

The main symptom is purpura which appears a short time after delivery Intracranial hemorrhage is reported in 20% with an immediate mortality of 12% and is the main clinical problem.

Thromboeytopenia is severe with a platelet count always less than 30 000/mm³ The bleeding time is prolonged, Coombs test is negative. The bone marrow reveals normal or increased number of megakaryocytes in most cases. In a *few* cases no megakaryocytes were seen in the marrow

In 50% of the suspected cases it has been possible to demonstrat isoantibodies against specific platelet antigens in the sera of th hematologically normal mothers. In all suspected cases the platelet incompatibility between the father and the child on one side and the mother on the other side has been established.

If the patient does not succumb to cerebral hemorrhage, spontaneous recovery can be expected in the course of 2 3 weeks

The treatment is under discussion. Good results are reported with pre and postnatal steroid medication and exchange transfusions.

Two cases, a sister and brother are presented. The mother was hematologically normal. The oldest patient died 4 days old from cerebral hemorrhage She did not receive any treatment The younger patient was treated with steroids and one exchange transfusion. On the 5th day no megacaryocytes could be seen in the bone marrow smear Eighteen days old the patient showed complete recovery No antibodies could be detected in the serum of the mother but incompatibility between the father s and the mother platelet antigens was demonstrated. The serologic tests were kindly performed by N R. Shulman, M D National Inst tut of Health, Bethesda, U.S.A

Gunnar Sidal Pyloric Stenosis

The material comprises 63 patients, of whom 25% girls, treated at Porsgrunn Lutherske Sykehus during the period 1954 to 1964. Thirty-eight % of the patients were submitted to the *Fredet Ramstedt* operation, while the remaining were given medical treatment Onset of symptoms occurred from the 1st to the 6th week of life. There was a clear tendency to earlier manifestation of symptoms as well as earlier admission to hospital for the patients who were operated. Palpable pyloric tumor pparent ventricular peristalsis, positive X-ray findings and dehydration were likewise far more frequent and marked in the patients who were operated. Four of the patients had icterus of unknown etiology on admission. The mortality was 3% as 2 patient died. Both had been operated, but the preoperative stat was poor

All patients primarily received medical treatment and surgery was only done if the medical treatment failed. In the surgically treated patients, the vomiting usually subsided about two days after the operation, whereas the tendency to vomit persisted for about three weeks in the medically treated. Duration of hospital st y was approximately 18 days shorter for the operated patients. Birth weight and weight on admission were similar in the operated patients, against a mean gain in weight of 233 g in the medically treated During a preoperative period a er aging 9 days, an actual loss of weight of 20 g occurred—this in a period where there should have been a physiological gain in weight of 235 g per week. On the other hand, the surgically treated patients had a far more substantial gain in weight postoperat ely

than did the medically treated during the corresponding period

In Norway the prevailing policy is to give medical treatment a trial in all cases and only if this procedure fails is operation undertaken. In the United States it has long been the custom to operate all cases of pyloric stenosis and in recent years surgical intervention has become the treatment of choice also in England Denmark and Sweden

From the own findings and the reports of other investigators, t is apparent that the medical therapy calls for considerably longer hosp talization and that improvement and gain in weight are slower than using surgical treatment. The protracted hospitalization and reduction of the patients general condition increase the risk of nosocomial infections. The possible mental consequences of a hospital stay of several weeks at this most impressionable age should likewise be taken into account

According to the majority of investigators, the medical therapy fails in about 50% of the cases which will usually mean that the operation is undertaken at a later and less favourable time than if t had been the primary treatment. In the countries where operation is the treatment of choice, duration of hospital stay is short, improvement occurs rapidly and mortality is no higher than using medical therapy

In the authors opinion, all patients with a definitely established diagnosis f pyloric stenosis should be operated Duration f pre- and postoperative medical therapy should be decided on the basis of the most opportune time for the surgical correction.

DISCUSSION

Arne Njå Regardless of personal opinions as to surgical treatment versus medical treatment of pyloric stenosis, I want to stress the point that children with this condition should be hospitalized. I ha e myself followed this rule for many years, but ha e been shocked and dismayed to see how often baby health stations and general practitioners will go on treating such patients for weeks on end regardless of the non-beneficial effect of the treatment. As a result, the patients have often been in a very poor condition when finally admitted to hospital. Such cases belong in hospital, and it is for the hospital doctors to decide whether or not medical treatment should be given a trial or whether operation should be performed t the soonest possible moment.

Karl W Wefring Megaloblastic Anaemia in Children

During the course of a family epidemic, a 20-month-old girl had gastroenteritis which persisted despite treatment. From time to time the diarrhoea was bloody and after 4 months she was admitted to hospital as a an spect ulcerative colitis. The main symptoms were anorexia, listlessness, emaciation and diarrhoea. She was markedly dystrophic and lost about 40% f her weight. There was ulceration of the buccal and vul al mucosa, Hb 58%, reticulocytes 2–3 per 1000 red cells. Marked anisocytosis. The bone marrow showed active erythropoiesis which was primarily regarded as normoblastic. A series of in estigations gave n further information. Despite blood transfusions and symptomatic treatment her condition changed ery little. A new bone marrow examination 6 months later showed definite megaloblastic erythropoiesis. There was a dramatic improvement on treatment with 10 mg folic acid daily After 2–3 days, her appetite returned, the faeces became normal and there was a reticulocytosis (p to 300 per 1000 red cells on the 6th day) with rising Hb The peripheral blood picture and the bone marrow became normal. In the restitution phase she had temporary oedema and enlarged liver which were interpreted as "nutritional recovery syndrome The treatment was stopped after 3 weeks and she has been well since (2 years).

Torbjörn Ström. Herpes Zoster in Infants

Varicella and herpes zoster are caused by the same virus. It is commonly accepted

that varicella is the primary reaction to the infection and that herpes zoster represents a subsequent clinical reaction in individuals with incomplete or reduced antibodies. A number of cases have been described in which an infant has developed herpes zoster without having had varicella and where no reasonable explanation for this has been demonstrable.

A case of herpes zoster in a girl aged ten years during an epidemic when all other children had varicella is reported. The girl had not been ill previously. It was revealed that the mother had had varicella about the fifth month of pregnancy. Therefore it seems a reasonable hypothesis that the girl developed herpes zoster as a primary reaction to the virus either because of presence of remnants of antibodies acquired from her mother or because the girl herself had had varicella in utero and thus been able to form incomplete antibodies.

Arve Haxeberg Uroscreen—a Chemical Test for the Detection of Bacteriuria

The test depends upon the ability of bacteria to reduce triphenyl-tetrazolium-chlorid (TTC) to red insoluble formazan. A positive test indicates significant bacteriuria (100,000 organisms per ml of urine or more).

The test has been used in 67 cases. When concentration of bacteria per ml urine exceeded 100 000 the test gave about 85 % positive results. In urine samples with no bacteriuria or less than 100 000 bacteria per ml there were about 5 % false positive reactions.

The test might be considered a useful screening method particularly in large-scale surveys.

Stein Høyer Deaths in the Neonatal Period

Clinical data and autopsy findings in some newborn infants who had died at the Children Department Porsgrunn Lutherske Sykehus, from fairly rare anomalies were demonstrated (cor biloculare, dextrocardia, ichthyosis congenita, hydroanencephalia seranía, diaphragmatic hernia, jejunal atresia, malrotation of the colon)

Peter Johan Moe Use of Human Growth Hormone in Hypopituitary Dwarfism

Four children at the Children's Hospital, University of Bergen, have received long term treatment with human growth hormone (HGH). Two siblings are presented. Both of them showed marked growth retardation from birth. They developed the typical appearance of pituitary dwarfism and the fasting blood sugar was low. Both failed to respond to intravenous metopirontest but they later on responded normally to oral metopiron test. Bone age was slightly retarded in the older one and normal in the other. Treatment with HGH 2 mg 3 times a week was instituted when the patient were $3\frac{1}{2}$ (♂) and 2 (♀) years old respectively. They have also received desiccated thyroid in order to secure full response to HGH. The treatment had to be discontinued temporarily for 2 months owing to untoward reactions and infectious disease in both of them. No side-effects to the treatment have been observed after re-institution of therapy. Both children have demonstrated a marked and steady growth rate during treatment. The growth rate decreased when the dose was reduced to 1 mg 3 times a week. Total growth during one year effective treatment was 17.5 and 15.5 cm respectively.

Meeting Sept 17 1965

W Blystad Neonatal Hypoglycemia

Blood sugar in premature and dysmature infants was examined using the Hultmann method. Samples were taken the first three days of life after 6 to 8 hours fasting.

In 57 prematures with birth weight from 850 to 2500 g the mean value was 31.8 mg/100 ml (12–119 mg/100 ml). There was no certain relation between low blood sugar and clinical symptoms.

A birth weight more than 20% below the mean for the infant's gestational age was used as the criterion of dysmaturity. Of 3800 newborn infants, 62 were classified as dysmature and in this group mean blood sugar was 54.2 mg/100 ml (10–125 mg/100 ml). Two infants had symptomatic hypoglycemia.

Over a period of 3 years we have in all observed 7 cases with cerebral symptoms in the neonatal period resulting from hypoglycemia. All of these were dysmature infant and in 6 cases pre-eclampsia was present in the mother.

Ö. Aagenaes. Hypoglycemia in Children

Hultmann's blood sugar method, which is currently employed at the University Hospital, Oslo, gives glucose values from 10 to 15 mg per 100 ml lower than Hagedorn and Jensen's method and the borderline between normoglycemia and hypoglycemia is at about 40 mg per 100 ml.

Some well defined enzyme deficiencies may cause hypoglycemia (glycogenosis, galactosemia, fructose intolerance, glycogen syntetase deficiency), but most of the hypoglycemias in older children may still be characterized as idiopathic. A not infrequent finding in the latter type is hypersensitivity to leucine—meaning that these patients present an abnormally high insulin increase following intake of leucine.

In late years some investigators have segregated a special type of hypoglycemia, marked by non-elevated epinephrine values in insulin induced hypoglycemia. However according to recent studies, this is a most unspecific response and, if the patient is tested shortly after a hypoglycemic attack, there will be no rise regardless of type of hypoglycemia. The diagnosis of insulinoma is a difficult one in children, but in hypoglycemias starting before 2 years of age it is very rare. Tolbutamide tolerance, which in adults represents a fairly good test, is of little differential diagnostic use in children.

Five cases of hypoglycemia are reported. The first signs of the illness were convulsions during infancy and the differential diagnosis was epilepsia. Two of the patients were markedly susceptible to leucine, two were moderately susceptible, and one showed a normal leucine reaction.

In the three patients submitted to insulin tolerance test, there was no rise in the epinephrine excretion in the urine. Two of these were markedly susceptible to leucine.

In the two cases where the susceptibility to leucine was moderate dietary treatment—frequent meals rich in carbohydrates and relatively low in leucine—had good effect. In the two cases where the hypersensitivity was marked, treatment with diet and corticosteroids proved beneficial. In the fifth case the hypoglycemia persisted despite treatment with corticosteroids, ephedrine, growth hormone and zink glucagon and the patient died in status epilepticus. Diazoxide was not tried, nor was exploratory laparotomy. The tendency to hypoglycemia usually decreases at about 5–6 years of age, but by that time about half of the patients will be mentally retarded.

Svein Oseid. Methods for Determining Blood Sugar and Insulin Activity

The current methods for determining blood sugar are summarized. Hultmann's method is described in more detail as this is now the standard method in the Pediatric Department, Oslo University Hospital. The method gives values that differ little from those obtained with glucose-oxidase tests. The main interfering substances are lactose, galactose, and mannose. The advantages of determining plasma sugar instead of whole blood sugar are discussed.

Account is given of an immunologic method for determining the insulin activity in plasma. The method has been described by Hales & Randle and is based upon an isotope dilution technique.

Gunnar Oftedal. Peritoneal Dialysis in Uremia in Children

The chief indications for using peritoneal dialysis in children are acute renal failure

with anuria/oliguria and life-threatening exogenous poisonings with or without kidney involvement.

Peritoneal dialysis is less effective per time unit than extracorporal hemodialysis, but can frequently replace the artificial kidney and is considerably simpler to practice in small children. Three cases in which peritoneal dialysis was carried out successfully are reported. The patients were a 20-day-old boy with uremia due to renal infection, a 3-year-old girl with possible hemolytic-uremic syndrome, and a 1 year-old boy who had developed uremia following gastroenteritis. The theoretical and technical aspects of and the complications associated with the peritoneal dialysis are summarized.

Meeting Oct. 22 1955

Arthur H Parmelee (Los Angeles): The Predictive Value of Developmental Testing in Infancy

J Steen Johnsen Experience from a Pediatric Missionary Hospital for Orphans in Korea

Meeting Nov 19 1965

Round Table Conference: Is the Preventive Health Scheme for Infants and Children in Need of Readjustment?

Participants. *J Steen A. Sundal, E. Ek*

Jens Steen My views can be summed up as follows: Every newborn infant has a claim to thorough medical examination immediately after birth. The doctor examining the infant should inform himself whether the mother has had any serious illness during pregnancy and whether there is any hereditary disease in the family *H should further see to it that these data are transmitted to the doctor who is to supervise the baby i future.*

During the first year of life, the child should be taken to medical control *three times* after which time the doctor should be able to tell whether the child is healthy or whether he is in danger of developing, or has already developed, handicap In the latter cases, close supervision is vidently called for

The working field of the public health nurses should be enlarged: If possible, they should examine the home conditions, and the baby at the end of the first month of life, and they should play a more prominent role at later controls. They should be able to advise the mothers on formulas and diets and to perform all accinations. They should further check that all infants are properly weighed and measured at least once a month. Finally they should take part in the subsequent follow-up, whether this takes the form of calling in all 3-year-old and 5-year-old children for a check-up, or of regular supervision based on an observation and handicap clinic and register for the district in which all children are examined, inclusive of those who during the first year of life have shown signs of illness or injury likely to prove a permanent handicap. From her records, the public health nurse should, at any time be able to tell how many of the babies born the previous year have met at the baby-health station and how many have been taken t a private doctor It should be her duty to look up the remaining and see to t that they are properly examined.

The goal should be a children s clinic in every district where handicapped and suspect handicapped children could go for bservation and thorough examination, where they could return for follow-up, and where the parents would receive the best possible professional advice Attached to these clinics should be specialists in psychology logopedics, and child pedagogics. A central committee for guiding and intensifying the work at the health st tions should likewise be established.

A. Sundal. It is now a little more than 50 years since the preventive health scheme for infants was introduced in this country. As is reasonable the main emphasis was placed on the prophylactic measures most needed t the time (artificial feeding of infants) and the lines along which it seemed opportune to proceed were mapped out accordingly. There is no doubt every reason to take up for revision the work covered by the health stations. I shall put the finger on some points that need to be changed:

1) The babies are taken to the first control too late. In 343 babies brought to the "well baby clinic" at the Pediatric Department University of Bergen in 1965, mean age at the first consultation was 10 weeks. If possible, the public health nurse should visit all mothers, say -3 weeks after deli very and incite them t take the baby to the health station or bring in a private practitioner should they prefer it.

2) An observation or handicap register as suggested by Dr Steen, would no doubt mark a great step forward. In Norway the majority of children are born in maternity departments and records covering the birth the examination of the infant in hospital, possibly also the first examination at a "well baby clinic" should be sent to the health officer of the district or to the State health department. On this department should rest the responsibility of providing adequate follow p and treatment facilities and sufficiently specialized staff. The health stations are hardly qualified to be in charge of an observation register.

3) In many part of the country there is not enough breast feeding and too much bottle feeding. This is associated with the fact that formula in addition to the breast milk is given too early and that it is left to the mother to increase the amount of formula as she sees fit. To change this is an important task for the health stations (doctor and assistant).

4) At present the health stations are pressed for time. There are too many consultations in the course of the day and the docto must ha e enough time for thorough exami nation. The frequency f consult tions should be decided upon in each individual case—one mother and child may be in strong need of returning at frequent intervals, while the intervals may safely be prolonged with regard to more robust types with good home conditions.

5) The doctor and public health nurse (or other assistant) form a medical team and are complementary to one another in the welfare work. The doctor must be the responsible party and should see all children who come in. In exceptional cases the doctor may delegate a consultation to his assistant, for instance if the health station is open only one day a month or so.

6) If the public health nurse, or assistant is qualified, she should perform vaccinations, tuberculin tests with reading, simple blood tests (Hb) and urine examinations.

Eyvind Ek. A health station should not be solely a control station, but a centre radiating welfare information in the broadest sense of the term to mothers to be and to mothers with children. Medical supervision during pregnancy is very important both from an obstetric and a pediatric point of view but the social aspects of the situation are of im portance too and, whenever possible help and guidance in this field should be given.

At the place of birth the mother should be instructed how to take care f her baby. Then, soon after coming home, she should be visited by the public health nurse because that is when the problems will hit her head on—especially if she is a primipara. Medical examination f the infant immedia tely after birth, preferably by a specialist, is of great importance. The proper steps can be taken in cases of prematurity possible anomalies, or other problems in the neonatal period.

At the health stations, it would be prefer able if every child could be examined by the doctor at every visit but due to the lack of doctors, this will not be possible. A quali fied public health nurse is able t relieve the doctor from some of the work. He would, however still be responsible and all children should be referred to him when the nurse finds it necessary. The nurse should be able to perform the vaccinations under the doctor responsibility.

Sven Ostad

Swedish Pediatric Society

Meeting Sept. 4 1965

Kurt Kayser The paediatrician and the last ten years of chromosome research

During the past 10 years, clinical advances in genetics have been of particular value to paediatricians. Studies of (1) sex determination, especially of newborn infants with pronounced hermaphroditism; (2) patients with other genital hypoplasias or malformations; (3) dwarfism and mental retardation; and (4) other malformations or degenerative diseases have been facilitated. In brief, the sex-chromatin and chromosome investigations have been very helpful for sex determination.

Gonadal dysgenesis is a condition where particularly the paediatrician comes in contact with the patient because of failure of onset of puberty.

Mentally retarded children who also have other malformations sometimes show chromosome aberrations. It is generally the paediatrician who must investigate such cases. Among these, he may find trisomies of the group 13–15 and 17–18 or abnormal occurrence of the number of X or Y chromosomes. An extraordinary discovery is trisomy 21 in mongolism. At last the etiological basis of this condition has been found and the paediatrician has good ground for diagnosis. However the cases of translocation trisomy or mosaicism in mongolism has complicated the picture and investigation of the parents of such children is also required.

Thus the paediatrician has gratefully accepted the advantage of improved diagnoses of many typical children diseases and expects in the future further advances in this clinical genetic field of research.

Bo Kjellström Prematurely born children in Eskilstuna

During the years 1950–64, there were 671 liveborn children with a birth weight of 2500 g or less at the Central Hospital (twins not included). The frequency of prematurity was 3.3% (including twins 4.1). Percentage mortality (during the first stay at the hospital) was 17.9%. Divided into different birth weight groups: 90.0% (0–1000 g), 62.6% (1001–1500) 22.1% (1501–2000) and 4.9% (2001–2500). The total number of liveborn children during the fifteen years has been about 20,600. Most of them were born during the spring (about 2000 per month). During the winter the same figure was about 1600 per month. However the frequency of prematurity was uniform throughout the year.

The mortality rate calculated for each month (totally during 15 years) was unequally distributed. The frequency increased at the end of the year. In March 10.8%, in December 28.9%. This does not correspond to an increase in the number of children born in the lower birth weight groups towards the end of the year. 120 prematurely born children died. 60 (= 50%) died during the first 24 hours, another 20 died the following day. About 90% died within the first four days.

During the years 1950–64, the average hospital stay for each surviving child was 33 days. This figure has decreased since the end of the fifties and has been about 23 days during the past 3 years.

Kurt Kayser and Alvar Johansson. Iris colour during the first year of life

According to general experience the colour of the eyes of a newborn infant gradually changes during its first year of life. The blue-eyed child of blue-eyed parents has its eyes change from a dark blue to a brighter shade while children who later become brown-eyed, initially have a dark blue iris.

Parents sometimes ask when eye colour changes from blue to brown. The doctor

answer is often vague because he doesn't know

For some years, we have taken serial colour photographs of the eyes of about ten children whose parents had either blue blue, blue–brown or brown–brown coloured eyes. Photographs were taken at birth and then every week or month during the first year of life. All of the children showed a change in eye colour. Of particular interest was the change from blue eyes initially to a browner colour.

Generally eye colour changed at 3 to 5 months of life. The investigations continue.

Kurt Kaijser: Collodium-baby

The term "collodium-baby" which is not very scientific has been used by many authors to describe a condition in which a child is born with a collodium like membrane around the body. This membrane disappears during the first weeks of life and the skin then looks quite normal.

A few such cases have been described during the past hundred years but the etiology remains uncertain. The question is whether this condition is a manifestation of true ichthyosis or some other skin disease.

There is some similarity between these membranes and a sort of membrane (epitrichium or periderm) which some newborn animal vertebrates have.

However in many cases, the condition has gradually changed to a real ichthyosis after the congenital membrane has disappeared. Presumably the different theories concerning the etiology could be reduced if it were accepted that collodium babies may subsequently develop different types of ichthyosis. (See R. H. Hermans "Kollodium babys" *Dermatologica*, *119* 164–185, 1959.)

An investigation of 3 collodium babies at the paediatric clinic in Eskilstuna shows that all had low birth weight and two had hydramnios. Furthermore, there was a positive family history of ichthyosis in two cases and all of the infants gradually developed a more typical ichthyosis after some months. One child was mentally retarded while a 2-year-old girl seems to be psychically normal. The youngest child is only 3 months old. In September 1963 a brother to the 2-year-old girl was born. Even this child is a typical collodium baby.

Kurt Kaijser: Synechia vulvae—urinary infection

Recurrent urinary infections in girls should lead not only to urography but also to investigation of the external genitalia to make certain whether there is any malformation of the urinary tract. Sometimes urinary disorders are related to fusion of the labia minora.

This is shown by the following case. A girl, 4½ years of age, has, during the last six months, had three episodes of frequent painful micturition. Prior to this she was well. She has been periodically treated with penicillin, sulfa and chloramphenicol. Proteus mirabilis and Streptococcus faecalis were cultured from the urine. The condition recurred and she was then investigated in a hospital with the following results: no albuminuria, moderate number of white and a few red blood corpuscles in the sediment, urography negative.

She was then sent to another hospital. Repeated urinalyses gave the same result as mentioned above. A careful examination showed synechia vulvae (fused labia minora). *Treatment*. Division of the labial fusion. She has had no recurrence of dysuria since then.

At the children's clinic in Eskilstuna, six cases of synechia vulvae have been seen during the last ten years. Obviously this condition is not so very rare. Nevertheless, even gynecologists are not always familiar with this condition.

In an American text book of pediatric gynaecology an author describes girls with fused labia who have been referred to plastic surgeons for a plastic operation for suspected vaginal atresia although a simple incision in the doctor's office is the adequate treatment.

The history of the above-described girl exemplifies the necessity for careful exploration of the external genitalia in females with urinary disorders.

Meeting Oct 9 1965

L Gamstorp B Kjellman and B. Palmgren
Diencephalic syndrome in infancy and childhood

Three unusual diencephalic syndromes in infancy and childhood are: (1) diencephalic emaciation syndrome (Russel 1951) (2) lipodystrophy and gigantism with hyperlipemia (Berardinelli, 1954; Seip 1959), and (3) cerebral gigantism (Sotos *et al* 1964)

One case of typical Russel syndrome and one case of the complet syndrome of cerebral gigantism are presented. In addition, the authors discuss 3 patients with symptoms of brain damage of different etiology but also with signs of a diencephalic lesion. All of the patients had large hands and feet.

Case 1 Girl, one year of age with complet emaciation syndrome (emaciation, locomotor overactivity euphoria) but also with remarkably large hands and feet The latter sign has not previously been reported in this syndrome. The patient had a chiasma-tumour (histologically verified optic glioma), as as the case in 23 cases reported in the literature.

Case 2 Girl, who was completely investigated three times between her second and fifth year of age because of acromegaloid gigantism, non-progressive neurological disease and advanced skeletal maturity—thus a complete syndrome of cerebral gigantism Serum SF activity (sulfation factor) was abov the upper 3 sigma level for age.

Case 3 Boy who had neonatal asphyxia and dysmaturity and who during infancy had accelerated increase in height to abov the upper 3 sigma limit In addition, he had remarkably large hands and feet and neomotor instability H soon developed a characteristic Hurler syndrome with skeletal changes, severe mental retardation, hepatosplenomegaly vacuolized lymphocytes, and urinary excretion of acid mucopolysaccharides.

Case 4 Boy 4 months of age, with generalized muscular hypertonicity emaciation, muscular hypertrophy and large hands and feet. He had neonatal asphyxia. Air-encephalography ... derate cerebral atrophy and ... anoxic brain lesion with dien... ement

Case 5 Gi... age with strabism, nysta... visual impairment and ... in spite of normal blood ... hands and feet—thus an incomp... ndrome Investigation, operat... mination disclosed an

Gunnar Mecanisme and Bertil L. dqu ...
The intermediate metabolism of he ... glucose-galactose malabsorption

In glucose-galactose malabsorption (GGM) the act ve transport of glucose and galactose across the intestinal mucosa is disturbed because of a genetic defect. Consequently the oral glucose tolerance test yields a more or less flat blood sugar curve Exceptionally however a blood sugar rise is seen with values up to 30 mg/100 ml which, of course may be misleading for the diagnosis. For contrast when loading these patients orally with fructose (30 g per sq m. body surface), the blood sugar may rise to 100 mg/100 ml, of which only about 20 mg/100 ml consist f fructose. Apparently patient with GGM are ble to con ert fructose t glucose Normal individuals, however after oral fructose loads, do not usually show an increase in blood sugar abov 30 mg/100 ml; the blood glucose level may even decrease a little Repeated oral fructose tolerance tests in patients with GGM indicate that the increase in blood glucose depends on the degree of carbohydrate star tion of the patients prior to the test. If patients with GGM are given normal part of their calorie supply as bsorbable carbohydrate (.e fructose) for some days before the test is carried out the result of the oral fructose tolerance test does not differ from that seen in normal individuals. Miller Craig Drucker & Woodward, studying normal individuals subjected to carbohydrate starvation, found that the intra enous fructose tolerance test resulted in higher increase in the blood glucose

value than that obtained when they were given a normal diet The ltered response of the blood glucose le el observed in patients with GGM as well as in normal indi iduals might be due to a decreased utilization of glucose (lowered glucokinase acti ity?) after carbohydrate starvation.

The elimination rate of intravenously in jected glucose also was found to be normal in GGM provided that a state of carbohyd rate starvation had been corrected. When these patient were placed on a low fructose diet (4 calorie per cent), the i v glucose tolerance test resulted in a curve of the diabetic type Thus, in this respect, patients with GGM do not differ from normal indi viduals subjected to carbohydrate starvation.

Patients with GGM eliminate intraven ously administered galactose more rapidly than glucose, just as normal individuals do. A possible relation of this response to the dietary carbohydrate intake was not studied

In summary these studies indicat that the intermediat carbohydrate metabolism is normal in glucose-galactose malabsorp- tion.

Tor Lindberg The prenatal development of dipeptidase activity in the gastrointestinal tract

F ve dipeptidase activities in the gastro- intestinal tract f 25 human f tuses between 11 and 23 weeks of age were determined by a spectrophotometric assay method. Studies of the development of the enzymes revealed that they were already well-developed at the fetal ge of 11 weeks. When studying the distribut on of the five dipeptidase activities along the gastrointestinal tract, t was found that the act ties were low in the stomach whereas they were high along the whole length of the small intestine In the large intestine the activities were of the same magnitude as in the terminal ileum. This contrast with the findings in the ad lt human, where the enzyme acti ties in the large intestine nd stomach are equally low

B. Hall. Follow up investigation of new born mongoloids with respect t growth retardation

How does the extra chromosome exert its action? Growth retardation in mongoloids has been suggested previously because of the similarity between newborn mongoloids *and normal fetuses. The extra* chromosome is thought to act as a diffusely disturbing factor which means that the *quantitative* rather than the qualitative genic content of the extra chromosome is significant ("the delay hypothesis") The similarity betw een the different autosomal trisomy syndromes points in the same direction. The syndrome with a larger extra chromosome (trisomy D) however exhibits more severe disturbances than those with smaller extra chromosomes (trisomy E and G) A larger extra chromo*some would be expected to cause early fetal* death. This idea, of course, by no means excludes the possibility that differences be- tween autosomal trisomy syndromes may also be related to the specific gene content of the extra chromosome.

A follow up investigation of newborn mongoloids and controls was done to see if the delay hypothesis is also valid *for postna- tal life. References* are, however made only to two easily measurable signs; head circum ference and length of the finger skeleton (dig. IV) Both in mongoloids and controls, these signs follow the same pattern: a short measure in the newborn period is partly compensated for by an increased postnatal growth. This agrees with the fact that there is greater variability in newborn mongoloids than in older mongoloids. Head circumfer ence and finger length of mongoloids are, however shorter t birth and continue to *increase at a slower rate than* in the controls. Fetal growth retardation is thus continued postnatally

Ingrid Gamstorp Gunnar Mossuasse and Nils Tryding Tryptophan loading test in convulsiv disorders

The tryptophan loading test was per formed in 60 children, 48 of whom had con *vulsions.*

Each child was given 100 mg L-tryptophan (Sigma) pe kg bodyweight; urine was collected over 7 hours, and the excretion of xanthurenic acid was determined with a fluorimetric technique after chromatography on the ion exchange resin Dowex 50–x8 100–200 mesh The result was xpressed as μmol xanthurenic acid per kg bodyweight excreted during 7 hours.

All children, with or without convulsions, not treated with hydantoin derivates, had values below 0.65. Of 30 children, wh took hydantoin alone or together with other an tiepileptic drugs, 17 had a value abo e 0.65. Most of these 17 children were tried on pyridoxine; none improved clinically Eight had a second tryptophane loading test, when they were on pyridoxine and un changed antiepileptic therapy The second test showed decreased excretion of xanthu renic acid in 6 and increased in 2.

So far only one child has had tryptophan loading tests both before and during anti con ulsive therapy The second test, per formed when diphenylhydantoin (10 mg/kg bodyweight) had been given for one month showed a slight increase in the excretion of xanthurenic acid.

Addition of diphenylhydantoin to urine samples did not interfere with the determi nation of xanthurenic acid.

Only one child was found to respond clinically to treatment with pyridoxine his tryptophan loading test was normal both before and during treatment with pyri doxine. This is in accordance with the result of other investigators studying the syndrome pyridoxine dependency

With the method used the results of the tryptophan loading test appears to be influenced by treatment with hydantoin derivates. Patients with pyridoxine depend ency may ha e a normal tryptophan loading test. A trial of pyridoxine and evaluation of the clinical and electroencephalographic response therefore ppears a better w y than the tryptophan loading test to find the patients, who may benefit f om the admini stration f pyridoxine.

Meeting Nov 7 1965

G. Koch and H Wendel: Comparison of pH, carbon dioxide tension, standard bicarbonate and oxygen tens n in capillary blood and in art rial blood during the neonatal period

The pH, pCO_2, standard bicarbonat and pO in arterial and capillary blood were compared at different ages during the neonatal period. All infants were full term with spontaneous ertex deli ery or deli ered by caesarean section. The majority had no signs of pre- or postnatal asphyxia or respiratory distress.

Capillary blood was sampled from the heel after hyperaemisation. Arterial blood was collected simultaneousl from an indwelling catheter in the umbilical artery Analyses were performed within 30 minutes after sampling, using Astrup micromethod and/ or pCO_2 electrodes. The material was divided into tw groups comprising the periods 30 minutes to 24 hours and 48 hours to 6 days.

Satisfactory concordance between arterial and capillary blood w found in the older age group with respect to pH, pCO an l standard bicarbonate wh le pO compares less favourably during the whole neonatal period.

It seems important to stress that this applies to infants with essentially normal central and peripheral circulation Judging from the results obtained in ad lt sh expected to be less f ourable in nfants wh respiratory distress and secondary impair ment of the circulation.

G Koch and H Wendel: Development of acid base regulation and blood gases in rmal infants during the first eek of life

In 40 normal infants, all full term with normal pregnancy and normal

very the development of acid base balance and of blood gases was studied during the first week of lif During delivery blood was sampled simultaneously from the umbilical artery and vein, further blood samples were obtained from an indwelling catheter in the umbilical artery Analyses were performed with Astrup's micromethod and/or pCO and pO electrodes.

The pH is low in umbilical artery blood at delivery and shows a further decrease in the minutes following delivery Within 30 minutes after delivery however the mean value is above 7.3 on the following days. Standard bicarbonate parallels fairly well the pH curve: the initial metabolic acidosis is rapidly reduced. It is only slight at the age of 4 hours and normalises during the following days of the neonatal period.

The initial CO_2-retention decreases rapidly and fter 60 minutes the mean value of pCO is less than 40 mm Hg. Thus, respiratory acidosis ppears to be restored within one hour after delivery

Oxygen saturation, initially very low increases rapidly reaching the same range as later in lif by bout 5 hours of age. Even pO increases rapidly after delivery the mean value however is significantly lower (70–80 mm Hg) than in healthy adults during the entire neonatal period There is, however marked interindividual variation.

Á Gyllensvärd and Mats Pehrson Osteogenesis imperfecta treated with sodium fluoride

Two children suffering from osteogenesis imperfecta were treated with sodium fluoride based on the findings in an American report of a similar case The first child had several fractures which resulted in shortening and deformities of the legs. H could still not walk at 3 years of age Six months later treatment with sodium fluoride ½ mg daily was started. During the following period, he learned to walk with the aid of crutches and had no fractures. Eight months after onset of therapy trauma resulted in a fissure in his right femur It was then learned that treatment had been discontinued two months previously Since then, he has not suffered any further fractures. After 10 months of therapy he can walk with slight support and moves fairly quickly on crutches. H is now receiving 5.5 mg of sodium fluoride daily

The second hild had a fracture of the right femur and pronounced bowing of the legs at birth. Treatment with sodium fluoride 3 mg daily was started at the age f ½ months. One year later the legs were less bowed and no fractures had occurred.

No side effects of the treatment were observed in either case. Further studies and more cases are needed before drawing conclusions about the efficiency of this treatment

L. Beckman, K. H Gustavson and A. Norring Dermal configurations in the diagnosis of the Down syndrome An attempt t a simplified scoring method

Deviations in the dermal configurations are known to be part of several syndromes caused by chromosomal aberrations. This applies especially for individuals with Down syndrome (mongolism), whose palm-print often show marked differences from those of normal individuals. A scoring method based on eight different dermal traits on palms and fingers has been devised, which provides a good separation of patients with Down syndrome from normal individuals. The probability that an in ividual with a certain score has Down's syndrome, has been estimated. The present investigation has shown that about half of the cases with Down syndrome diagnosed cytogenetically could have been safely diagnosed by means of a dermatoglyphic analysis. The possibility for diagnostic laboratories to meet the increasing demand for cytogenetic diagnosis by using a combination of dermatoglyphic and cytogenetic methods has been discussed. (Published in *Acta Genet.*, 15 3, 1965.)

B. Hellström, K. Thorell and U Sallmander: How regularly are prescribed medicines taken in cases of epilepsy in children?

By means of interviews, data was obtained on 158 epileptic children examined at Karolinska Hospital to ascertain how regularly the prescribed t blet treatment was followed. In 12% of these cases, it was found that the medication was not being taken entirely regularly and in 8% it was less satisfactory. Recurrent ttacks in connection with failure to take the medication were observed in some cases. No definite relationship could be found between the degree of regularity and certain variables, such as social group duration of the epilepsy frequency of attack, and so on. The incidence of irregularity in taking prescribed tablets is certainly a mini mum value, as no other method was used to control this information. The results of this investigation ha e been published in more detailed form elsewhere

B. Werner I Rune and B Wengle Difficul ties in the dietary treatment of phenylketonuria

W th reference to a 22 month-old boy with phenylketonuria who had been treated since the age of 4 months some pitfalls in the dietary treatment of phenylketonuria were discussed.

The boy was t the time of discussion normally developed as far as could be deter mined from the general condition psychological tests and EEG

During the treatment the patient ga evidence of two of the most common diffi culties: the risk for phenylalanine deficiency in early life and the inst bility of the phenylalanine level during infection.

The phenylalanine deficiency was not detected until the method for phenylalanine determination was hanged. One-dimensional paper chromatography had been used. This gave erroneously high levels of serum phenyl alanine leading to the patient being kept on too low administration of phenylalanine for t least 4 months, but with adequat calorie intake A fluorimetric method disclosed the deficiency the plasma values being as low as 0.4–0.5 mg/100 ml. Despite the comparatively long period of deficiency the blood concentration remained low and not as might ha e been xpected from reports in the lit rature high due to breakdown of body protein. During periods of infection, how ever the plasma alues rose to bnormal levels and this happened both in lighter attacks which did not influence the intake of food and in more severe ones which did so.

The clinical picture in severe deficiency was quit similar to that in infection. When in doubt as to which of these was involved determination of phenylalanine in urine was of importance In phenylalanine deficiency there was an aminoaciduria without phenylalanine excretion (–dimensional paper chromatography) In infection there was an in creased excretion of phenylalanine without general aminoaciduria.

The importance of urinary as well as blood assays is pointed out as is the necessity of carrying out repeated assays during periods of infection. Infections should be trea ted act ely to shorten them whenever possible One-dimensional paper chromatography may lead to erroneous conclusions. Methods giving reliable quantitation also at low phenylalanine concentrations must be used. Good laboratory facilities are a prerequisit for adequat treatment

DISCUSSION

L. Hambraeus, R Lorentsson and L. Wranne A two-year-old girl was admitted to hospital with phenylketonuria which had been diagnosed at the age of one year Since then she has been on a low phenylalanine diet On admission, the plasma phenylala nine alues were abnormally high The mother stated that the patient s ppetit had been poor during the last few weeks and that she refused to eat the prescribed foods. As t was suspected that the patient had nega t e protein balance and that this had resulted in catabolism of endogeneous protein, her metabolism balance was in estigated.

During a trial period of 7 days, she was

given a diet containing 2 g of protein (including 15 mg phenylalanine) and 100 kcal per kg bodyweight per day. She was then put on a diet for 10 days with a protein content of about 10% of the previous one with essentially no change in the phenylalanine and calorie content. Daily fluorometric determinations of the plasma phenylalanine concentration were done using the method described by Wong *et al.* (1964). The diagram shows a rapid increase to 15 mg/100 ml on the ninth day. Following readministration of 2 g of protein per kg bodyweight per day the plasma phenylalanine concentration reverted to normal or subnormal levels within two days. Urinalysis showed that the urinary phenylalanine concentration did not accurately reflect the concentration of this substance in the plasma during the trial period, the values of phenylacetic acid, phenylpyruvic acid and o-hydroxyphenylacetic acid being within the normal range.

This case suggests that in patients with phenylketonuria who are on a diet which provides insufficient amounts of protein for their nutritional requirements, endogeneous protein is catabolized and this results in overload of phenylalanine. It is therefore of major importance that patients with this disease are carefully followed by checking protein and phenylalanine levels in their diet.

B. Hellström, K. Theorell and Birgitta Jalling: Agenesis of the corpus callosum

In connection with a report of five cases with this anomaly treated in the Pediatric Department at Karolinska Sjukhuset, a review was given of the functions of the corpus callosum based on clinical observations and experiments in animals. The five cases mentioned resembled clinically those described in the literature, with mental retardation as the main symptom. It was particularly interesting that in one case there was a dissociation between the EEG activity of both hemispheres, with a highly pathological hypsarythmia-like activity from the left hemisphere and completely normal activity from the right hemisphere. In another case there were periodic hyponatremia. The results of this investigation will be published in more detailed form elsewhere.

Meeting March 12, 1966

O. Fi nström: Wiskott Aldrich's syndrome

Wiskott Aldrich's syndrome, which is characterised by thrombocytopenia with a bleeding tendency, increased disposition to infection, especially bacterial infections, as well as eczema, was first described by Wiskott in 1936. Aldrich *et al.* showed that the syndrome was of recessive, sex-linked, hereditary nature.

Two brothers, six months and 18 months old, with the typical features of this disease, were examined in the Department of Pediatrics at Umeå. There was no family history of this disease. The older brother suffers primarily from bleeding, while the younger has eczema and increased tendency to infections.

The thrombocytes varied between 10–150,000. Bleeding-time was prolonged, coagulation-time normal. Clot retraction-time was prolonged and prothrombin consumption increased. Thrombocyte antibodies could not be shown. The results speak in favour of increased bleeding tendency caused by thrombocytopenia.

Investigations of the bone marrow showed that normal thrombocyte agglutination around the megakaryocytes was absent. There was also eosinophilia and a decreased number of plasma cells.

The Mantoux test was negative, inspite of BCG vaccination. Serum electrophoresis was essentially normal. Immuno electrophoresis with quantitative determination of different gammaglobulin fractions showed an increased IgA fraction. The younger brother had a low level of serum isoagglutinins, which is typical; while the elder brother had a high

tier. Passive cutaneous anaph   is with milk as the antigen was posit   Neither child could be shown to have ad   l tissue in the epipharynx on roentgenol   l examination.

P Köhlin. K tones and hypogl ce

Eight children with pontan   idiopathic hypoglycemia have been   iagnosed at the department of pediatric   Lund. The clinical picture agrees w th   hat described by Colle & Ulstrom (*J Pediat 64* 632, 1964). All the children were comatose on admission. They had ketonuria, hypoglycemia, and regained consciousness q ickly   administration of intra enou   glucose A ketogen-rich diet (cream-casein mix ure   00 kcal/m² divided into 4 portion   was gi en at 8, 11 14 and 17 hours) precipitated symptoms of hypoglycemia in these children including apathy frequently   weating, well as a low blood sugar (12 23 mg 100 ml) within 24 hours. Administration of glucose rapidly relieved these symptoms. Intra enous insulin and tolbutamide loading produ ed pathological decrease in blood sugar   alues, with clinical symptoms of hypoglycemia, as well as delayed or no-return to fasting values. An increase in pulse and blood pressure was observed during these loading tests.

In one child with hypoglycemia due to leucine sensitiv ty a ketogen diet provocation test could not be carried out because the patient became hypoglycemic after the first meal, in contrast to the findings of Colle & Ulstrom.

Mebane & Madison (*J Lab Clin Med 63* 177 1964) ha e shown an increase in insulin like activity and decreasing blood sugar and FFA values on intra enous   dministration of acetic acid and β-hydroxybutyric acid in dogs. In order to determine whether there was an increased tendency to hypoglycemia in children with spontaneous hypoglycemia on administration of ketones, N   β-hydroxy butyrate   v   us   dose of 1 mmol/kg body weight was given. In the fi e cases hitherto in estigated the blood sugar decreased an a erage of 1   7 23) mg/100 ml during the first 20–30 m   tes after injection. In 3 normal children administration of a similar dose resulted in a decrease in the blood sugar of 0–5 mg/100 ml) during the same period. The pathogenesis of these findings was discussed.

G Koch. Veno-arterial shunting during the neonatal period. I. Findings in normal newborns

Healthy newborns show a relat   arterial hypoxemia with an alveolar-arterial pAO – paO difference of about 20–25 mm Hg Alveolar hypoventilation as a cause of this hypoxemia can be excluded on the basis of pCO   which during the entire neonatal period less than 40 mm Hg Among other possible causes, ventilation perfusion imbalance and red ced diffusion can be eliminated by in spiration of 100% oxygen and complete   ashout of nitrogen. In these circumstances,   persistent pAO – paO difference solely depend on a "true veno-arterial shunt   The location of this shunt (whether cardiac or intrapulmonary) cannot however be determined.

In 31 f ll term newborns, oxygen tension (paO_2) after 20 minutes inspiration of 100% O   was determined during the first week of life. The total veno-arterial shunt was calculated by means of the classical shunt-formula on the basis of an arterio-   enous oxygen difference of 35 ml O_2/l blood. Arterial blood was obtained with a polyethylene catheter in the umbilical artery

From the age of 5 hours, the paO   was 400 mm Hg in all cases, and the corresponding shunt was < 20% of the cardiac output. The   verage paO ranged from 500 to 550 mm Hg, while the shunt ranged from 10% to 15%. At the time of the first examination (20–50 min tes after deli ery), the   verage paO   was considerably lower and the ranges for the paO and shunt were 100 to 300 mm Hg and 38% to 15 ., respecti ly The result of the investigation therefore show that   t least one cause of relative hypoxemia in   newborn is veno-arterial

shunting which from 5 hours of age is probably confined to the lungs. This method seems particularly suitable for evaluation of the degree of pulmonary atelectasis and of right-to-left shunt in infants in whom measurement of functional circulatory parameters is particularly difficult

G. Koch and H. Wendel. Veno-arterial shunting during the neonatal period. II. Clinical studies

For the past year we have been carrying out serial determinations of oxygen tension in arterial blood and the effect of inhalation of a high percentage of oxygen, as well as determining the acid base status in cases of respiratory insufficiency in newborns. By this means it was possible to obtain a more complete understanding of the functional state of the lungs and lesser circulation.

Four cases of different types of initial respiratory difficulties were demonstrated: (1) Aspiration syndrome (shunt 50% and 85%). Patient died after 24 hours. (2) Very premature infant with kernicterus (normal lung function and 23% shunt). Patient died after one week. (3) Hyaline membrane disease (shunt 26). Patient survived. (4) Marked hypovolemia with severe acidosis, pH 6.80 (shunt 30, subsequently 20%). Patient survived. In cases where the arterial oxygen tension exceeds 100 mm Hg on inhalation of a high percentage of oxygen, the prognosis appears to be relatively good. This corresponds to a shunt of less than 40% of the minute volume

Britt Fredriksson and S. Sjölin. Hospital meals for children

The consumption of calories, protein, fat carbohydrate iron and vitamins on two week-days was determined in 30 children aged 6 to 16 years who were patients in the department of pediatrics at Umeå Hospital. None of the children was seriously ill or was suffering from nutritional disorders. All of them were up and about during most of the day

The average calorie consumption was considerably lower than the normal values quoted for healthy children. The low calorie consumption probably resulted from reduced physical activity and poor appetite consequent on sickness. On certain days the unappetising nature of the food definitely played some role. The total calorie break down into protein fat and carbohydrates showed that the amount of fat was significantly higher than what is recommended −42% as compared with the 30–35% recommended. Intake of protein, iron, niacin, vitamins A, D and C was, in most cases, considerably lower than the recommended daily requirement for healthy children. The main reason for this was probably the low calorie consumption, but also partly a poor choice of menu. The meals were poorly served and unappetising

Requirements for more protein and less fat can easily be satisfied by changing the diet. To ensure that hospitalised children receive adequate amounts of vitamin C fruit or juice with a high vitamin C content should be given daily Neither vitamin A nor vitamin D requirements can be satisfied entirely by diet, especially since the fat intake must be reduced Supplementary ingestion of these vitamins is therefore necessary It is uncertain whether diet can supply an adequate amount of iron. It may prove necessary also to give iron supplements.

P. G. Bergfors. Hereditary elastodystrophy

Hereditary elastodystrophy (HE) is characterised by degenerative changes in the elastic connective tissue. The symptoms appear in the skin (pseudoxanthoma elasticum), and the heart vessels (weak or impalpable peripheral pulses, gastro-intestinal bleeding that is difficult to stop, generally increased bleeding tendency etc.). Hypertension is often present.

Skeletal changes seem to occur remarkably often, and are generally associated with elevated values of acid phosphatase in the serum. Apophysitis in particular should be searched for (type Schlatter Scheuermann, etc.).

A boy now 14 years old, had already as an infant an increased tendency to bleed, and was given iron for anaemia on several occasions. At the age of 10 he was hospitalized for persistent melena which endangered his life for nearly two weeks. At the same time he was also found to have hypertension and a localized rash on the neck. Biopsy showed this to be pseudoxanthoma elasticum.

The patient has since developed other symptoms suggestive of hereditary elastodystrophy barely palpable radial pulses, characteristic ocular fundal changes, heart enlargement, rising blood pressure (now about 175/120) a cyst in the right tibia, bilateral Schlatter disease as well as elevated acid phosphatase. In 1964, he had a recurrence of severe malena. His subjective symptoms are limited to a tendency to epistaxis and sharp pains in the chest.

In 1964, his sister aged 11 died of heart attack while skiing. Autopsy showed an enlarged heart. On histological examination, the endocardium and myocardium, as well as several valves, showed typical hereditary elastodystrophy changes.

Two cousins died at the age of about two, and had at that time severe hypertension (250/150). Autopsy showed nothing remarkable apart from cardiac hypertrophy. Hereditary elastodystrophy changes were also present on histological sections.

Another cousin who died at the age of four of renal vein thrombosis previously exhibited deafness, defective balance, tendency to convulsions and mild mental retardation. The clinical picture may perhaps be explained by hereditary elastodystrophy changes in the intracranial vessels, but no microscopic evidence of hereditary elastodystrophy was found in the few tissue preparations which were preserved from this case.

There is a family history of consanguinity.

T. Berg and Kerstin Malm. Idiopathic pulmonary hemosiderosis.

Two cases of idiopathic pulmonary hemosiderosis are described. One of the patients, a girl, was taken ill in 1964 at the age of ten months. In 1966 when the patient was 20 months old, prednisolone treatment was started. The patient, who at the introduction of the treatment was critically ill, improved dramatically within a few days. Cardiac catheterization was done when the patient had improved and showed normal pressures and saturations in the pulmonary circulation. The second patient, also a girl, fell ill in 1960 and since 1961 she has been treated with prednisolone with an apparently favourable effect on her disease. For some length of time it has been possible to give the prednisolone treatment intermittently. Cardiac catheterization in 1965 showed normal pressures and saturations and normal pulmonary vascular resistance.

Rutger Lagercrantz

ANNOUNCEMENT

The Department of Pediatrics at the University of Wisconsin Medical Center, Madison, Wisconsin, will sponsor three-day Post-Graduate Conference on "The Mentally Retarded Child." Guest lecturers at the conference to be held April 6, 7 and 8, 1967 will be Dr. Leon Eisenberg, Professor of Child Psychiatry, Johns Hopkins University Medical School; Dr. Lionel S. Penrose, Director of the Kennedy-Galton Centre, Hertfordshire, England; Dr. Julius B. Richmond, Professor of Pediatrics, Upstate Medical College, State University of New York; Dr. Arthur J. Lesser, Associate Director of Children's Bureau, Department of Health, Education and Welfare; Dr. David Y. Hsia, Professor of Pediatrics, Northwestern University Medical School; Dr. Charles F. Barlow, Neurologist-in-Chief, Harvard Medical School.

Inquiries regarding attendance at the conference should be addressed to Harry A. Waisman, M.D., Professor of Pediatrics and Director, Joseph P. Kennedy, Jr. Laboratory, University of Wisconsin Medical Center, 1300 University Avenue, Madison, Wisconsin 53706.

NEW BOOKS RECEIVED

Books received by *Acta Paediatrica Scandinavica* are acknowledged under this heading. Selected books will be reviewed in subsequent issues space permitting.

L. I. Woolf: Renal Tubular Dysfunction. Amer. Lecture Series. Charles D. Thomas, Springfield, Illinois, 1966. 252 pages, ill. Price: US$9.50

D. Y. Yung Hsia: Inborn Errors of Metabolism, Heart I. Clinical Aspects. Yearbook Medical Publishers, Chicago, Illinois, 1966. 396 pages, ill. Price: US$11.00.

Handbuch der Haut- und Geschlechtskrankheiten, II. Band/2. Teil: *Entzündliche Dermatosen II.* Springer Verlag, Berlin–Heidelberg–New York, 1965. 921 pages, ill. Price: DM 338.

M. Cornblath and R. Schwartz. Disorders of Carbohydrate Metabolism in Infancy. Volume III in the series: *Major Problems in Clinical Pediatrics.* W. B. Saunders Company. 1966. 297 pages, ill. Price: £2. 19s 6d

F. Geubelle: Contribution a l'étude fonctionelle du poumon de l'enfant sain et de l'enfant asthmatique. Editions J. Duculot, S. A. Gembloux 1966. 235 pages, ill. Price: 450 francs belges.

H. Opitz und F. Schmid. Handbuch der Kinderheilkunde. Springer Verlag, Berlin–Heidelberg–New York, 1966. II. Band/1 Teil: *Pädiatrische Diagnostik.* 952 pages, ill. II. Band/2 Teil: *Pädiatrische Terapie.* 785 pages, ill. Price (both volumes): DM 468.

BOOK REVIEW

G. Andrews and M. Harris: The Syndrome of Stuttering

Clinics in Developmental Medicine No. 17. W. Heinemann Medical Books Ltd, London 1966. 183 pages. Price: £2 or US$6

More than one percent of the school children in Western Europe stutter. Stuttering is found among all peoples and is not restricted to any specific nation or culture: about the same percentage of stutterers is found in all language areas investigated. These findings are related in the opening chapter of the volume. The book is an account of a major study on stutterers made by the authors together with several other specialists in Newcastle upon Tyne during the years 1962–1964. We still know very little about the problem of stuttering but this research makes a very positive contribution. The investigation was made among a group of stuttering school children aged 9 to 11. All school teachers were asked to report on all cases of stuttering. These children were then interviewed by speech therapists who attempted to determine the extent and type of stuttering in each child. 86 children were then classified as true stutterers. Of these 80 were able to take part in the investigation and were matched against 80 non-stuttering children of the same age, sex, intelligence and social background. Their parents, especially the mothers, also attended psychiatric and general interviews but not at the same time as the children. The authors were surprised to find that the stutterers were not more emotionally disturbed than the non-stutterers. The study also shows that the stutterers began talking at a later age and came from less stable environments. Another characteristic was the generally subnormal intelligence of the stutterers and their mothers. It should be mentioned that the average total time given to each case was 3.5 hours. A more thorough investigation might have produced slightly different results for some of the variables. The final part of the book deals with an investigation of the syllable method of reading as a therapeutic aid in treating stuttering. The initial results appear to be favourable.

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